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Annual Report of Intramural Activities

October 1, 1986 - September 30, 1987

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

National Institute
of
Allergy and Infectious
Diseases

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U.S. Department of Health and Human Services
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TABLE OF CONTENTS

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1987 ANNUAL REPORT OF INTRAMURAL ACTIVITIES

INTRAMURAL RESEARCH PROGRAM

OFFICE OF THE SCIENTIFIC DIRECTOR........................1-1

ANIMAL CARE BRANCH.......................................2-1

ADMINISTRATIVE MANAGEMENT BRANCH.........................3-1

BIOLOGICAL RESOURCES BRANCH..............................4-1

LABORATORY OF CELLULAR AND MOLECULAR IMMUNOLOGY..........5-1

LABORATORY OF CLINICAL INVESTIGATION.....................6-1

LABORATORY OF IMMUNOGENETICS.............................7-1

LABORATORY OF IMMUNOLOGY.................................8-1

LABORATORY OF IMMUNOPATHOLOGY............................9-1

LABORATORY OF IMMUNOREGULATION..........................10-1

LABORATORY OF INFECTIOUS DISEASES.......................11-1

LABORATORY OF MICROBIAL IMMUNITY........................12-1

LABORATORY OF MOLECULAR MICROBIOLOGY....................13-1

LABORATORY OF PARASITIC DISEASES........................14-1

LABORATORY OF VIRAL DISEASES............................15-1

ROCKY MOUNTAIN LABORATORIES

LABORATORY OF MICROBIAL STRUCTURE AND FUNCTION........16-1

LABORATORY OF PATHOBIOLOGY............................17-1

LABORATORY OF PERSISTENT VIRAL DISEASES...............18-1

OPERATIONS BRANCH.....................................19-1

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1987 ANNUAL REPORT PROJECT NUMBER LISTING*

Z01 AI	
00013-24	LIP
00020-12	LVD
00027-20	LMM
00030-19	LI
00035-12	LI
00036-22	LI
00043-22	LCI
00045-19	LCI
00047-18	LCI
00048-17	LCI
00057-14	LCI
00058-13	LCI
00071-16	LPB
00072-16	LPVD
00074-15	LPVD
00082-26	LPB
00085-10	LPVD
00086-10	LPVD
00094-28	LPD
00097-29	LPD
00098-31	LPD
00099-17	LPD
00102-13	LPD
00103-20	LPD
00108-16	LPD
00123-21	LVD
00126-14	LVD
00131-20	LMI
00134-25	LMI
00135-13	LIP
00136-15	LMI
00138-13	LIP
00143-18	LMI
00144-23	LMI
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00162-11	LPD
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00173-10	LIG
00180-09	LIG
00182-09	OSD
00183-09	OSD
00186-14	LI
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00190-09	LMM
00192-09	LCI
00193-08	LMSF
00197-08	LPD
00199-08	LPVD
00201-08	LPB
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00205-07	LIP
00208-07	LPD
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00266-06	LPVD
00269-06	LCI
00271-06	LCI

*Does not include terminated or inactive projects

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1987 ANNUAL REPORT PROJECT NUMBER LISTING

Z01 AI
00272-06 LCI
00275-06 LCI
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00368-05 LID
00370-05 LID
00372-05 LID
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00389-04 LIG
00390-04 LIR
00391-04 LVD
00392-04 LVD
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00403-04 LI
00404-04 LID
00405-04 LID
00406-04 LID
00413-04 LMSF
00415-04 LMM
00416-04 LVD
00417-04 OSD
00418-04 LPVD
00423-04 LMI
00425-03 LI
00427-03 LI
00428-03 LCI
00429-03 LCI
00430-03 LCI
00431-03 LIR
00432-03 LCI
00433-03 LMM
00434-03 LMM
00437-03 LMM
00438-03 LMM
00439-03 LPD
00440-03 LID
00441-03 LMSF

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1987 ANNUAL REPORT PROJECT NUMBER LISTING

Z01 AI
00442-03 LMSF
00443-03 LVD
00445-03 LVD
00446-03 LID
00447-03 LCI
00450-03 LID
00451-03 LID
00457-03 LID
00459-03 LID
00465-02 LIP
00466-02 LMM
00467-02 LMM
00468-02 LPVD
00469-02 LCI
00470-02 LCI
00473-02 LID
00474-02 LID
00475-02 LID
00476-02 LID
00477-02 LID
00478-02 LID
00480-02 LPB
00481-02 LCI
00482-02 LCI
00483-02 LPD
00484-01 LIP
00485-01 LCMI

Z01 AI
00486-01 LCMI
00487-01 LPD
00488-01 LPB
00489-01 LPB
00490-01 LPB
00491-01 LPB
00492-01 LPB
00493-01 LI
00494-01 LPD
00495-01 LCI
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00508-01 LID
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00510-01 LID
00511-01 LID

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OFFICE OF THE SCIENTIFIC DIRECTOR, NIAID
1987 Annual Report
Table of Contents

Z01-AI Project Number		Page
Summary of Program		1-2
00417-04	Systematics and Vector Relationships of Ticks (Ixodoidea)--Keirans	1-3
00172-09	Synthesis of Peptide Antigens--Maloy	1-4
00182-09	Biochemical & Genetic Mechanisms of Obligate Intracellular Parasitism	1-5
00183-09	Immunologic Properties of *Coxiella Burnetii* (Q Fever) Vaccine	1-6

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Summary of Program
Laboratory and Clinical Research, NIAID
October 1, 1986-September 30, 1987

The Intramural Research Program (IRP) of the National Institute of Allergy and Infectious Diseases consists of 14 Laboratories. The individual descriptions of research in these laboratories are contained in the following pages. Eleven of the laboratories are located at the NIH campus in Bethesda, MD. Several Sections from the Bethesda laboratories, as well as contract support services, are at NIAID facilities at the Frederick Cancer Research Facility, Frederick, MD. The remaining three laboratories are located at the Rocky Mountain Laboratories (RML) in Hamilton, Montana. The Office of the Director, IRP (ODIRP), is responsible for the management of the entire Intramural Research Program.

This past year several significant changes occurred within the NIAID IRP. Dr. Norman P. Salzman, Chief, Laboratory of Biology of Viruses retired and the laboratory was disestablished. In addition, it was decided that the Laboratory of Microbial Immunity would be phased out and the various components were assigned elsewhere within the IRP. Two new programs were created within NIAID, IRP. The Biological Resources Branch (Dr. John E. Coligan, Branch Chief) was created to centralize and provide vital services for members of the IRP. The Laboratory of Cellular and Molecular Immunology (Dr. Ronald H. Schwartz, Laboratory Chief) was also established to expand the IRP's effort in basic immunology.

In addition to the above changes NIAID IRP has had considerable growth during FY 87 with a 19.14 percent increase in the appropriated budget to an annual level of $72,425,000. Additionally, the IRP received 30 FTE positions bringing the NIAID IRP ceiling to 550. The total IRP staff (FTEs and nonFTEs) currently is 752 persons. Most of this increase in resources was for new activities for research on AIDS. To meet these needs an AIDS Vaccine Development and Treatment Center was established. The Center was created to support the research of NIAID IRP scientists and contains an AIDS Virus Molecular Microbiology Unit (Drs. Malcolm Martin and Bruce Chesebro), an AIDS Clinical Immunology Unit (Drs. Anthony S. Fauci and H. Clifford Lane), an Antiviral Agent Unit (Drs. Lane and Martin), a Vector Development Unit (Dr. Bernard Moss), and an Animal Model Unit (Drs. Robert Chanock, Robert Purcell, Herbert C. Morse and Bruce Chesebro). Additional space was identified and is currently under renovation at the Frederick Cancer Research Facility (4000 sq ft) and in Rockville, MD (26,550 sq ft) for new IRP staff and contract personnel to support the center. Renovation of Building 4 started this year. The renovation is currently on schedule with the anticipated move from Building 5 to Building 4 scheduled for the summer of 1988. The total amount of space in all the NIAID IRP increased in FY 87 by 18.4 percent, from 165,484 sq. ft. to 196,034 sq ft.

NIAID animal facilities at RML and Frederick meet AAALAC standards. Considerable progress has been made to bring the facilities at Bethesda up to standards and major renovations of animal facilities will begin shortly at the Bethesda campus.

Many outstanding accomplishments towards understanding AIDS were made by NIAID scientists from numerous IRP laboratories, including LIR, LMM, LVD, LIG, LIP, and LPVD. Some highlights of this year's studies include epidemiological studies showing that the risk of infection following percutaneous injury or mucosal splash with AIDS virus infected material is less than 1.0%. In addition, persistence of antibodies to the gag protein of HIV as well as HIV-specific IgM responses may be associated with favorable prognosis. Other observations include development of a transgenic mouse model to study the early stages of AIDS, demonstration of the mononuclear phagocyte system as the major cell type supporting HIV replication in the brain, demonstration that azidothymidine (AZT) may be of value in the treatment of early stages of HIV infection, and generation of monoclonal antibodies specific for the envelope protein of HIV which can be used to detect infected cells by live cell membrane immunofluorescence.

A major focus of IRP investigations in LID and LVD has been on the delineation of the etiology, pathogenesis and epidemiology of medically important viral diseases and the development of means for their control. As outlined in this report, the application of new molecular biology technology has resulted in considerable optimism that a number of important viral pathogens, such as hepatitis A virus, respiratory syncytial virus, parainfluenza type 3 virus, dengue virus and rotavirus, will soon be prevented. The development of new attenuated live virus vaccines or use of viral protein expressed in a recombinant vector, such as vaccinia virus, are major approaches currently under development by NIAID scientists.

NIAID IRP investigators in LPB continue to develop both synthetic and recombinant candidate vaccines against Bordetella pertussis. An important component of Bordetella pertussis, the S1 subunit, has been expressed in E. coli and animal protection studies using recombinant material have begun. Studies of other bacterial diseases by the LMSF have resulted in the molecular cloning and sequencing of genes from four different serotypes of Chlamydia trachomatis that encode the respective organism's major outer membrane protein. In other studies an animal model of trachoma has been developed in which chlamydia-infected guinea pig eyes are treated with penicillin to produce rapid opacification of the cornea; this model should greatly facilitate efforts to develop a vaccine against trachoma. Studies of mouse scrapie agent by LPVD resulted in expression of a cDNA clone of the mouse scrapie-associated prion protein gene. When this prion protein was inoculated into mice, it failed to induce scrapie, suggesting that mouse prion protein alone is not the infectious scrapie agent, as had been thought by many investigators.

Although the malaria sporozoite vaccine trial by LPD was disappointing in terms of efficacy of the vaccine, the experience led to important insights and new approaches to evaluating immunogenicity of synthetic peptide and recombinant antigens. In other studies LPD investigators initiated an innovative program in vector research to make mosquitoes refractory to malaria. In addition, the first transgenic mosquito was made. Another accomplishment has been the identification of the parasite receptor for binding to the Duffy blood group antigen on red cells, a potential target for vaccine development.

NIAID scientists in the LI, LIR, LIG, LIP and LCI remain world leaders in basic immunologic research and in studies of other aspects of host defenses and allergic diseases. The application of molecular biology techniques to these systems has resulted in many exciting advances which are outlined in the annual reports from each laboratory.

Equal Employment Opportunity and Affirmative Action Programs have been given considerable attention by the Office of the Director, IRP. Conscious efforts to recruit minorities at the professional level have been initiated. The NIAID IRP-OD Introduction to Biomedical Research Program was held in February as usual. This year we were also able to renew our program for Summer Students and over 75 students representing colleges from throughout the country participated; 40 of the summer students selected were from minorities.

The NIAID Board of Scientific Counselors, (Dr. Robert B. Couch, Chairman) reviewed the Laboratory of Immunology, Laboratory of Immunoregulation, Laboratory of Immunogenetics, Laboratory of Immunopathology, and Laboratory of Microbial Immunity. The high quality of scientific research in the IRP continues to be recognized in these evaluations. The Board supported the administrative changes outlined above. This year Drs. Teruko Ishizaka and Roy Patterson completed their terms on the Board and the IRP has been particularly appreciative of their contributions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00417-04 OSD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Systematics and Vector Relationships of Ticks (Ixodoidea)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James E. Keirans	Research Entomologist	OSD, NIAID
OTHERS:	R. G. Robbins	Museum Specialist	OSD, NIAID

COOPERATING UNITS *(if any)* Dr. C. E. Yunker, Heartwater Project, Harare, Zimbabwe, Drs. D. Sonenshine and P. Homsher, Old Dominion Univ., Dr. J. B. Walker, Onderstepoort, R.S.A., Dr. R. Pegram, Tick Diseases Unit, Lusaka, Zambia.

LAB/BRANCH

Office of Scientific Director

SECTION

Entomology Department, Museum Support Center, Smithsonian, Wash., D.C.

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The activities of this research project currently comprise three main functions: (1) Identification of ticks received from various individuals, universities and governmental agencies throughout the world. This is the only laboratory in the world capable of performing this function; (2) Systematic investigations of the Ixodoidea including taxonomy, classification, and life histories; and (3) entry, retrieval and manipulation of our tick data base on the Smithsonian computer system to provide researchers with the latest updated information on any tick species.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00172-09 OSD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Synthesis of Peptide Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	W. Lee Maloy	Section Head	BRB, NIAID
Others:	John E. Coligan	Branch Chief	BRB, NIAID

COOPERATING UNITS *(if any)*

Thomas Kindt, LIG, NIAID; Louis Miller, LPD, NIAID; Ronald Schwartz, LI, NIAID; David Margulies, LI, NIAID, Ethan Shevach, LI, NIAID.

LAB/BRANCH
Biological Resources Branch

SECTION
Synthetic Peptide Antigen Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	1.0	1.8

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Within the last year we have synthesized over 300 peptides for use in 50 separate studies. In collaboration with David Margulies's lab (LI), antipeptide sera reactive with the C-terminus of murine major histocompatibility complex class I molecules have been used to investigate alternative RNA splicing patterns in various mouse strains and cell types. In collaboration with the laboratories of John Coligan (BRB), Ron Schwartz (LI) and Ethan Schevach (LI) antipeptide sera have been used to identify a second T cell receptor for antigen in the mouse. We have followed the ontogeny of the new receptor, investigate the assembly of the receptor complex and identified which subsets of T cells express the receptor. The assembly of the primary T cell receptor complex on human T cells has been studied with antipeptides sera which are specific for the individual proteins of the complex. In work done in collaboration with Louis Miller's Lab (LPD), synthetic peptides have been used extensively to study the malaria parasite and the immune response it generates. Three histidine rich proteins present in the infected erythrocyte have been characterized. We have also defined the immunodominate epitope on the circumsporozoite protein; in both human and rodent malarias. In addition to the definition of B cell epitopes on the circumsporozoite protein we have also used synthetic peptides to define T cell epitopes in the human malaria Plasmodium falciparum. By conjugating the immunodominate B cell epitope with the major T cell epitope we have constructed a synthetic immunogen capable of generating an antisporozoite response in mice unresponsive to either peptide separately. In collaboration with Ron Schwartz's lab we have defined a helper T cell epitope in the cytochrome C molecule. Using this knowledge we have made a set of synthetic peptides where the proposed T cell receptor contact amino acids are changed. Each member of this set produced a specific T cell response when injected in mice, indicating that there are no "holes" in the T cell response to this epitope.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00182-09 OSD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Biochemical & Genetic Mechanisms of Obligate Intracellular Parasitism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
J. C. Williams, PhD, Sr Scientist, NIAID/OSD, Chief, Rickettsial Diseases Laboratory, USAMRIID
M. H. Vodkin, PhD, USAMRIID
T. F. McCaul, PhD, Sr Fellow, National Research Council Fellow

COOPERATING UNITS *(if any)*
K.-I. Amano, Hirosaki University School of Medicine, Hirosaki, Aomori, Japan
V. N. Reinhold, Department of Biological Chemistry, Harvard Medical School, Boston, MA

LAB/BRANCH
Office of the Director of Intramural Research Programs, NIAID, Bethesda, MD

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project was the cloning, expression and sequencing of a major antigen gene and to further chemically characterize the lipopolysaccharide (LPS) of Coxiella burnetii, the etiological agent of Q fever. A. Gene cloning. A gene library from the DNA of C. burnetii has been constructed in the cosmid vector pHC79. An immunoreactive gene product from clone pJB196 was identified as a 62 kilodalton (Kd) polypeptide both by Western blot and an in vitro transcription, translation system. Expression of the 62 Kd and a 14 Kd polypeptide was under the control of a heat shock promoter (HSP) in recombinant E. coli. Subcloning of pJB196 and sequencing a 3 Kb stretch of that DNA revealed two open reading frames, encoding a polypeptide of 58.9 Kd, and 10.5 Kd along with two appropriately spaced ribosomal binding sites. A transcriptional control element observed on the 5' side of the initiation codon resembled a HSP (at -452). Termination of this bicistronic mRNA synthesis may be under control of a rho-independent terminator with double dyad symmetry and ΔG of -28.0 and 43.5 K cal, respectively. Four sequences were highly homologous to the 62 Kd protein from C. burnetii (≥ 50%). Three are from Mycobacteria and represent the immunodominant antigen of this species. The other is from Escherichia coli, detected as a gene that complements or suppresses a temperature-sensitive RNAase activity. Therefore this protein is conserved in phylogenetically distant bacterial genera. B. LPS Structure. The core structure of C. burnetii LPS consists of (1-3)-mannoheptose backbone with every residue (1-2) branched with a single mannose moiety. Lipid C (the counterpart of Salmonella lipid A) is quite unusual in that it lacks carbohydrate and is small in molecular weight. C. Significance. A heat shock operon in C. burnetii produces a major antigen highly homologous to a protein in both Mycobacteria and Escherichia coli. The core structure of LPS was defined and the lipid C molecule is significantly different from Salmonella lipid A.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00183-09 OSD

PERIOD COVERED
October 1, 1986 to September 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Immunologic Properties of Coxiella burnetii (Q fever) Vaccine

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*
J. C. Williams, PhD, Sr Scientist, NIAID/OSD, Chief, Rickettsial Diseases Laboratory, USAMRIID
G. H. Scott, PhD, USAMRIID
D. M. Waag, PhD, USAMRIID
C. R. Bolt, MS, USAMRIID
T. F. McCaul, PhD, Sr Fellow, National Research Council Fellow

COOPERATING UNITS *(if any)*
G. R. French, DVM, and Staff, The Salk Institute, Government Services Division Swiftwater, PA

LAB/BRANCH
Office of the Director of Intramural Research Programs, NIAID, Bethesda, MD

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
4.5	2.5	2.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Immunogenic and pathogenic subfractions of Coxiella burnetii, the etiological agent of Q fever, are being studied in animals and humans to evaluate humoral and cellular mechanisms of immunity. A. Mice. Phase I whole cell and chloroform-methanol residue vaccines prepared by Salk Institute protected A/J mice from lethal challenge by aerosol and intraperitoneal routes. The aerosol route did not induce antibodies against phase I lipopolysaccharide, yet marked increases of antibody against surface proteins were measured. This surprising result contradicts the traditional view that antibodies against LPSI are a measure of immunity. Therefore protein subunit vaccines may be efficacious. The resistance of C. burnetii to formalin treatment has led to the evaluation of gamma (^{60}Co) dose response to inactivate residual viable organisms in vaccine preparations. One thousand kilo Rads are required to kill 3.8×10^{10} organisms per ml of medium for the effective sterilization of vaccines and reagents. B. Immune Modulation. The immune suppresive complex (ISC) virulence factor was expressed differently among 9 strains of C. burnetii. Strains expressing only smooth LPSI carried the most potent ISC. None of the strains with only rough LPSII expressed the ISC. However, one strain isolated from heart valve did not express the ISC although it contained both smooth and rough LPS chemotypes. C. Antigen Shift. Immuno-gold labeling techniques were used to verify that the different morphologic forms of C. burnetii express different antigenic surfaces. Also the endospore within the large cells were not detected by any antibody preparation. D. Seroepidemiology. A case controlled study to determine the incidence and risk for acquisition of Q fever has revealed that parturient cats are significant in outbreaks of atypical pneumonia. The role of cats in the spread of Q fever to humans may impact significantly of this zoonosis. E. Significance. This project identified a non-toxic efficacious Q fever vaccine, derived a gamma irradiation protocol to kill residual C. burnetii, described different components of the ISC and the distribution among strains, verified antigenic shift among cell variants and defined the role of parturient cats in the spread of Q fever.

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ANIMAL CARE BRANCH
1987 Annual Report
Table of Contents

Summary..2-1

LCI
LIG
AMB
BRB
LCMI

ADMINISTRATIVE MANAGEMENT BRANCH
1987 Annual Report
Table of Contents

Summary..3-1

ADMINISTRATIVE MANAGEMENT BRANCH

National Institute of Allergy and Infectious Diseases

October 1, 1986 to September 30, 1987

The Administrative Management Branch was established within the Office of the Director, IRP in June, 1986. The Branch was established to provide (1) planning, directing and coordinating of the administrative management functions for the IRP, including personnel management, financial management, space management, procurement, and other administrative functions necessary to ensure the efficient and effective implementation of Program objectives; (2) program planning guidance and assistance to the Director, IRP and Lab Chiefs in their respective areas; (3) assistance in the design, implementation and coordination of administrative and related automated information systems for the program.

During the past year the Branch has been very active in establishing new and improved systems to streamline Program efficiency. Procedures have been established for budget reviews, personnel reviews, EEO/promotion/award monitoring, and joint endeavor/collaborative agreement review. A "tickler" system was established that provides information on upcoming due dates and is updated on a quarterly basis. A tracking system was also developed to monitor the use of the Visiting Fellow and IRTA positions to assure appointments do not exceed the assigned ceiling.

The Branch and its staff have also coordinated the establishment and implementation of an NIAID-wide local area network computer system. This task included the procurement of all equipment, the cabling of buildings geographically separated, the selection of appropriate software, the design of a menu driven program for access and use of LAN software packages and development of special programs for internal use. The special designs include programs for space management, the performance management system and a laboratory data manager that assists in the procurement and budget tracking processes. The Branch assisted in the establishment of an NIAID Computer Training Center currently located in Bldg. 7. The center is set up with its own LAN to assure that staff is trained on the use of an LAN as well as the specific software packages such as WordPerfect, D-Base III plus and Lotus. The training center will also provide assistance with the programs that have been specifically developed for internal use by NIAID staff.

With the official establishment of the Branch, the staff has been subdivided into Administrative Teams located in the various IRP buildings located on and off campus. Each administrative office includes and Administrative Officer, Administrative Technician, purchasing agents and a facility manager. These offices are located in Bldgs. 10, 7, 5 and the FCRF. The day-to-day operations have been reorganized into specific areas of concentration such as budget, contracts, etc. and staff has been reassigned to head up these areas. The reorganization allows for improved expertise and efficiency within these target areas. Due to the increase in budget, space and personnel the AMB has expanded to include additional procurement staff and an Administrative Officer responsible for all IRP contracts. The IRP is striving to set up a procurement system that can respond to the needs of program staff and adhere to the rigid regulations required by the Federal Acquisition Regulations and related DHHS, PHS, and NIH policies.

In order to enhance the work environment, renovations have taken place in the administrative areas of Bldgs. 10, 5 and 7 to improve working conditions, eliminate excess noise and provide more efficient use of space. This has allowed the staff to expand without the need for additional space and has even resulted in a 8% loss of space assigned to the AMB. Quarterly seminars have also been initiated for the AO's and procurement staff to improve work habits and provide career development. Training has included time management, interpersonal group relations, stress management and procurement.

The Branch has also overseen the renovation and construction of Bldg. 4 on campus and Bldgs. 550 and 468 at the Frederick Cancer Research Facility. These renovations will result in additional space for the IRP labs as well as improved working conditions for many of the laboratory staff.

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BIOLOGICAL RESOURCES BRANCH
1987 Annual Report
Table of Contents

Summary..4-1

BIOLOGICAL RESOURCES BRANCH

The Biological Resources Branch investigates the structure and function of the genes and molecules involved in the immune response, the molecular basis for antigen presentation and recognition, and hemapoietic cell differentiation. In addition to this fundamental immunological research, the Branch provides protein sequencing, peptide synthesis and flow cytometry expertise and services for the NIAID.

T cell receptors. Multiple molecules exist on the surface of lymphocytes that are important for development, activation and regulation of the immune response. Of prime importance in this regard is the clonotypic αß heterodimer T cell receptor (TCR) that is noncovalently associated with the multicomponent T3 complex. In order to study the expression of the TCR-T3 complex, rabbit anti-peptide antibodies specific for human TCR α and ß chains, and T3 δ-chains were prepared. With these reagents, it was possible to show that all of the α, ß and CD3 chains present on the surface of lymphocytes are bound as a TCR-CD3 complex and that the two ß chain isotypes (Cß1 and Cß2) were expressed in about equal amounts. In the mouse an anti-peptide antibody was used to define a completely new TCR-T3 complex. This was initially described on Lyt2$^-$, L3T4$^-$ thymocytes and was found to consist of a 35 Kd gamma chain disulfide linked to a 45 Kd δ chain. This receptor is expressed prior to the αß TCR in the developing thymus and appears to be representative of a distinct T cell lineage differentiating within the thymus. Additional studies detected this receptor on a novel population of Thy-1$^+$, T3$^+$, Ia$^-$ dendritic epidermal cells (DEC). At least 60% of these cells were positive upon in situ staining with the anti-gamma chain antibody. Cell lines derived from DEC were found to express three types of novel TCRs: "classical" gamma-δ of 35 Kd and 45 Kd respectively, an apparent gamma-gamma homodimer and an as yet undescribed receptor that may contain $C_{\text{gamma}}4$ protein. Hybridomas that express these receptors and that can be grown in large amounts are being used to analyze the structural and functional properties of these receptors.

Variability in class I molecular structure. Major histocompatibility complex encoded class I molecules are integrally involved the presentation of viral antigens to cytotoxic T lymphocytes (CTL). The goal of these studies is to gain an understanding of the regulation of expression, and the functional and antigenic properties of these molecules. Research has focused on the fact that multiple class I molecules can be generated from single genes by alternative splicing of pre-mRNAs. The alternative class I forms vary in expression in different cell types and in their ability to undergo phosphorylation. It is tempting to speculate that alternative splicing may influence function by controlling the phosphorylation of class I molecules. The complexity of the murine H-2D region was further underlined by the discovery of additional D-region encoded molecules in the H-2^q haplotype and the delineation of a new family of D-region molecules which suggests a strong role for unequal crossing over in the evolution of D-region genes. Multiple species were examined for the expression of soluble class I molecules equivalent to the murine Q10 molecule. Only members of the order Perrissodactyla were found to have such a molecule suggesting that independent evolutionary events created class I genes of similar function. In the human, the cloning and sequence analysis of the HLA-A11 gene revealed that the close antigenic relationship of HLA-A3 and HLA-A11 was founded in their high structural homology and was likely related to a shared glutamine residue at position 62. From this data, a predicted cross-reactive recognition by CTL between the rare HLA-A3 subtype, HLA-3.2, and HLA-A11 was confirmed and offered support for the critical role of amino acid positions 152 and 156 in CTL recognition of class I molecules.

Hematopoietic cell differentiation. The use of neoplasms as model systems for hematopoietic cell differentiation has been well established. A wide variety of in vitro and in vivo derived tumors induced with the ecotropic MuLV, Cas-Br-M, with retroviruses containing the oncogenes ras, bas, fes, src, and abl, or with chemical agents have been studied. These studies have been complimented with experiments involving transfer of normal cells into mice with severe combined immunodeficiency disease (SCID). It was found that, contrary to current views of hematopoietic cell development, the B cell and the myeloid lineages may share a common progenitor cell and that this cell may be identified by the expression of Ly-17 (Fc gamma receptor) and Mac-1, an antigen thought to be restricted to the myeloid lineage. In addition, studies of T cell lymphomas induced with the Cas-Br-M MCF virus, NS-6, and the SCID mice experiments suggest that Mac-1 may also be present on T cell progenitors. Two very different model systems have been utilized to study the changes in bone marrow hematopoiesis associated with perturbations in the periphery. Inoculation of mice with the anemia-inducing drug phenylhydrazine was shown to stimulate B, T and myleoid cell differentiation in addition to the production of erythroid progenitors. In contrast, infection with the LP-BM5 immunodeficiency-inducing virus induces profound changes in pre-B and B cells but does not effect the development of other lineages in the bone marrow.

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Laboratory of Cellular and Molecular Immunology
1987 Annual Report
Table of Contents

Z01-AI Project Number		Page
Summary		5-1
00485-01	T Cell Activation	5-4
00486-01	T Cell Differentiation	5-5

PHS-NIH
Summary Statement
Office of the Chief
Laboratory of Cellular and Molecular Immunology
November 18, 1986 through September 30, 1987

Introduction

The Laboratory of Cellular and Molecular Immunology was created in November, 1986. Its major task is to perform research on thymus-derived (T) lymphocytes. Its objective is to understand how these critical cells of the immune system differentiate and function. The approach is to define problems in whole animal systems, set up *in vitro* analogs in tissue culture, and determine the molecular basis for the phenomena. The laboratory employs techniques from the disciplines of cellular immunology, molecular biology and protein biochemistry.

During its first year the laboratory has pursued three major areas of research: 1) The molecular aspects of antigen presentation, 2) The biochemical basis of T cell inactivation, and 3) The definition of a new T cell lineage, the T cell receptor (TCR) $\gamma\delta$-bearing cell.

Molecular Aspects of Antigen Presentation

The laboratory has defined two T cell antigenic determinants from pigeon cytochrome *c* at the molecular level by using synthetic peptides with single amino acid substitutions. This approach identifies residues that interact with the T cell receptor (epitope) by eliciting unique T cell clones specific for the substituted amino acid. Changes at residues that interact with the Ia molecule (agretope), do not elicit new clones, but are affected by changes in the allelic form of the Ia molecule. After mapping out the functionally relevant sites, multiple substitutions were made at the epitope to probe the diversity of the T cell repertoire. Surprisingly very few holes were found in the repertoire, suggesting that the major site of Ir gene control may be at the level of peptide-Ia molecule interactions. (B. Fox, K. Ogasawara, K. McCoy and R. Schwartz LCMI/NIAID).

The Biochemical Basis of Inactivation of T Cell Clones

In the process of quantitating the role of Ia molecules in the activation of T cells using purified Ia molecules in planar lipid membranes, we discovered that this form of antigen presentation inactivated normal T cell clones. A similar state was induced if normal antigen-presenting cells were first treated with the chemical cross-linker, 1-ethyl-3-(3 dimethylaminopropyl)- carbodiimide (ECDI) prior to antigen presentation.

During the induction of this unresponsive state, the T cells enlarge, make interleukin 3 and interferon-γ, but fail to synthesize interleukin 2. This state is induced solely by a rise in intracelluar calcium, as it can be mimicked by exposure of the cells to the calcium ionophore, ionomycin. Once in this state, the T cell clone can no longer produce interleukin 2 in response to normal activation signals (M. Jenkins, D. Mueller, G. Jung and R. Schwartz LCMI/NIAID).

Discovery of a New T cell lineage bearing TCR γδ.

During the past year our laboratory, in conjunction with several other laboratories at NIH, has identified the murine protein encoded by the T cell-specific gamma gene. This rearranging gene encodes part of a receptor on a new T cell lineage having a CD4 ,CD8 phenotype. The gamma chain is associated with another protein, termed delta, to which it is disulfide-linked on the cell surface. TCR γδ-bearing cells are the earliest T cells to arise in ontogeny, proceeding the TCR αβ cells by 2-3 days. They then migrate to the periphery where they are found in small numbers in the spleen and lymph nodes and form the predominant population in the epidermis of the skin. These cells produce lymphokines such as IL-2 when stimulated with lectins or anti-T3 monoclonal antibodies and are activated to kill certain target cells in a non-MHC-restricted manner. The normal physiologic function of these cells and the ligands they recognize are currently under investigation (D. Pardoll and R. Schwartz LCMI/NIAID; B.J. Fowlkes LMI/NIAID; A. Lew and J. Coligan LIG/NIAID; R. Germain LI/NIAID; J. Bluestone IB/NCI; and A. Kruisbeek BRMP/NCI).

Honors, Awards and Scientific Recognition

During the past year Dr. Schwartz was awarded the Meritorious Service Medal by the U.S. Public Health Service for "exceptional performance and contributions to modern research in cellular immunology and immunogenetics." He is a member of the editorial boards of Science, The Journal of Molecular and Cellular Immunology and Immunology Today. He was an invited speaker at the Workshop on Immunological Tolerance in Basel, Switzerland, the annual meeting of The American Society for Cell Biology in Washington, DC, the annual meeting of the Federation of American Societies for Experimental Biology in Washington D.C., a symposium on Self-Nonself Discrimination at the University of Kentucky, in Lexington, Ky, the UCLA Symposium on the T Cell Receptor in Keystone, Colorado, a meeting on Theoretical Immunology in Santa Fe, New Mexico, the annual meeting of the American Cytology Society in Cambridge, England, a workshop on T Cell Activation in Health and Disease in Oxford, England, and a symposium on T Cell Induction and Autoimmunity in Birmingham, England.

Dr. Jenkins was an invited speaker at the annual meeting of the Federation of American Societies for Experimental Biology in Washington D.C and at the FASEB summer research conference on Lymphocytes and Antibodies at Saxton's River Vermont. Dr. Pardoll was an invited speaker at the UCLA Symposium on the T cell receptor in Keystone Colorado, the European Immunology Network workshop on T Cell Ontogeny in Les Embiez, France, and the annual meeting of the Federation of American Societies for Experimental Biology in Washington, DC. Dr. McCoy was a minisymposium speaker at the annual meeting of the Federation of American Societies for Experimental Biology in Washington, DC.

Administrative, Organizational and Other Changes

During the past year Drs. Zdenko Kovac and Kristian Hannestad and Ms. Gina Stievenart joined the laboratory and Drs. Barbara Fox, Kasumasa Ogasawara, Ms. Cindy French, and Ms. Grace Jung left the laboratory after completing their training.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00485-01 LCMI

PERIOD COVERED

November 18, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

T Cell Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.H. Schwartz	Chief	LCMI, NIAID
Others:	M. Jenkins	Staff Fellow	LCMI, NIAID
	B. Fox	Guest Worker	LCMI, NIAID
	K. Ogasawara	Visiting Fellow	LCMI, NIAID

COOPERATING UNITS *(if any)*

T. Chused and J. Mizoguchi LI, NIAID

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
5 1/2	3 1/2	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Stimulation of IL-2 producing T cell clones with antigen and Ia molecules on chemically-modified antigen-presenting cells (APC) resulted in long-lasting proliferative unresponsiveness to subsequent antigen stimulation with normal APC. The induction of this unresponsive state was accompanied by little or no IL-2 production, although some lymphokines were suboptimally produced. The critical biochemical event appeared to be an increase in intracellular calcium. This system should be useful for analyzing the molecular events related to T cell tolerance induction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00486-01 LCMI

PERIOD COVERED

November 18, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

T Cell Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.H. Schwartz	Chief	LCMI, NIAID
Others:	D. Pardoll	Medical Staff Fellow	LCMI, NIAID
	D. Mueller	Medical Staff Fellow	LCMI, NIAID
	K. McCoy	Staff Fellow	LCMI, NIAID

COOPERATING UNITS *(if any)*

B.J. Fowlkes LMI, NIAID; A. Kruisbeek BRMP, NCI; A. Lew and J. Coligan LIG,NIAID, J. Bluestone IB, NCI, R. Germain LI, NIAID

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4 1/2	3 1/2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have identifed the product of the rearranging T cell receptor gamma chain. The gamma chain is disulfide bonded to a partner protein, termed delta. The gamma-delta heterodimer is found on a small population of cells in both the thymus and periphery. It is the first T cell receptor to appear during T cell development and defines a distinct T cell lineage whose development parallels that of classical TCR$\alpha\beta$-bearing cells. In contrast to TCR$\alpha\beta$-bearing cells, however, there is a very limited diversity of expressed receptors within the population of TCR $\gamma\delta$-bearing cells from a given strain.

PHS 6040 (Rev. 1/84)

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LABORATORY OF CLINICAL INVESTIGATION
1987 ANNUAL REPORT
TABLE OF CONTENTS

Z01-AI Project Number		Page
Summary		6-1
00043-22	Immunology and Chemotherapy of Systemic Mycoses--Bennett	6-12
00045-19	Studies on Interaction of Antibody and Complement on Production of Immune Damage--Frank	6-13
00047-18	Clinical Studies of Patients with Known or Suspected Parasitic Diseases--Ottesen	6-14
00048-17	The Pathophysiology of Autoimmune Hemolytic Anemia--Frank	6-16
00057-14	Basic Studies on Pathogenic Fungi--Kwon Chung	6-17
00058-13	The Pathogenesis and Chemotherapy of Herpesvirus Infections in Man--Straus	6-19
00154-12	Events in Immediate Hypersensitivity--Kaliner	6-20
00155-12	Phagocytic Cell Function--Gallin	6-21
00189-08	Clinical and Biochemical Studies of Human Enteral Adenovirus Infections--Straus	6-22
00192-09	Studies on Immediate Hypersensitivity--Metcalfe	6-23
00249-06	The Pathogenesis, Diagnosis, and Treatment of Systemic Mast Cell Disorders--Metcalfe	6-24
00250-06	Clinical and Basic Studies on Inflammatory Diseases of the Gastrointestinal Tract--Metcalfe	6-25
00269-06	Neutrophil Subpopulations--Seligmann	6-26
00271-06	Purification and Characterization of Serum Complement Proteins and Fragments--Hammer	6-27
00272-06	Host Defense Against Pneumococcal Bacteremia--Frank	6-28

Laboratory of Clinical Investigation

Z01-AI Project Number		Page
00275-06	The Complement Receptor and C3 Mediated Opsonization--Gaither	6-29
00276-06	Studies of the Membrane Attack Complex of Human Complement--Joiner	6-30
00277-06	Mechanism of Serum Resistance in Bacteria--Joiner	6-31
00278-06	Studies of the Components of the Complement Cascade--Hammer	6-32
00279-06	Studies on Mucous Glycoproteins--Kaliner	6-33
00354-05	Immunoregulatory Defects in Inflammatory Bowel Disease--James	6-34
00355-05	Immunoregulatory Defects in Primary Biliary Cirrhosis--James	6-35
00356-05	Studies of the Regulation of IgA Immunoglobulin Synthesis--Strober	6-36
00357-05	Studies of the Autologous Mixed Lymphocyte Reaction--Strober	6-37
00396-03	Complement Receptors: Regulation of Expression and Cell Biology--Malbran	6-38
00397-04	Interactions of C3b with Immunoglobulin G-Regulation of C3b Function by Antibody--Fries	6-39
00398-04	Studies on the Interaction of Complement and Parasites--Joiner	6-40
00428-03	IgE Immunotoxins--Kaliner	6-41
00429-03	Studies on Nasal Responses--Kaliner	6-42
00430-03	Molecular Biology of Varicella-Zoster Virus Infections--Straus	6-43
00432-03	Regulation of Mucosal Immune Responses In Non-human primates--James	6-44
00447-03	Clq: Its Biosynthesis and Biological Functions --Frank	6-45

Laboratory of Clinical Investigation

Z01-AI Project Number		Page
00469-02	Complement: Studies in Viral Infection --Fries	6-46
00470-02	Sporadic Neurasthenia Associated with Epstein-Barr Virus	6-47
00481-02	Receptors and Transduction Mechanisms in Human Phagocytes--Malech	6-48
00482--02	Endothelial Cells in Inflammation--Rotrosen	6-49
00495-01	Biochemical Events in Mast Cell Secretion--Kaliner	6-50
00496-01	Interactions between the Human Immunodeficiency Virus (HIV) and Herpesviruses--Ostrove	6-51

Summary of Report
LABORATORY OF CLINICAL INVESTIGATION
October 1, 1986 to September 30, 1987
Michael M. Frank, M.D., Chief of Laboratory
and Clinical Director, NIAID

INTRODUCTION

The last year has seen a return of the LCI to full strength with additional staff joining Dr. Harry Malech's group. The year has also seen a flowering of research productivity in all sections of the laboratory with a renewed emphasis on clinical programs and at the same time a continuation of programs in the basic sciences as they relate to our individual areas of investigation. The group has remained quite stable over this period of time with no major changes in Senior Staff. Nevertheless, there have been major changes in research program with a renewed emphasis on Molecular Biology, and the use of recombinant DNA technology to answer questions in medical science. The major emphasis of the group is still on problems in host defense and immunology and we still have three major areas of investigation. The allergy program consists of the sections of Dr. Michael Kaliner, head of the Allergy Section and Dr. Dean Metcalfe, head of the Mast Cell Physiology Section. The Immunology portion of the program consists of the Clinical Immunology Section under the leadership of Dr. Michael Frank and the Mucosal Immunity Section under the leadership of Dr. Warren Strober. The Infectious Disease Group consists of the Bacterial Disease Section under Dr. Harry Malech, with John Gallin a senior member of that section, the Mycology Disease Section under Dr. John Bennett, and the Viral Disease Section under Dr. Steven Straus. All groups attempt to work together although our experimental techniques and patient populations differ. To further increase coorperation between units as well as to increase critical analysis of on going programs this past year we had the first, two day retreat of members of the Senior Staff. We presented our experimental programs as well as our clinical programs and opened them for criticism by members of the Senior Staff. Resulting from the discussion has been a renewed interest in each others programs among members of the Senior Staff with an increasing level of collaboration. What is more important, renewed vitality of the clinical program has been noted as a result of these efforts. Members of our staff continue to be recognized as important contributors to many aspects of medical research with frequent invitations to write chapters in major textbooks, to attend international meetings, to participate in programs that lead to decisions about future directions of funding, etc. and even to participate in radio and TV programs.

Bacterial Disease Section

The bacterial Disease Section is head by Dr. Harry Malech. This is Dr. Malech's first complete year as member of the Laboratory of Clinicial Investigation and it is reasonable to consider the direction and substance of the group and how it has changed in the year since he has taken the helm. The focus of the Bacterial Disease Section has clearly moved in a direction of increased number of molecular approaches with a great increase in the use of molecular biological techniques to probe neutrophil function. Both surface receptor function and biochemical bases for phagocytic cell function are being studied. Using the high affinity label technique the group was able to show that the formyl peptide chemotactic receptor on polymorphonuclear neutrophils contains both complex and high mannose type polysaccharides and that it appears developmentally in the late pronormoblastic stage of myeloid differentiation. New subtypes of Gi regulatory proteins that affect cell function were shown to exist on myeloid cells and the subtypes of Gi were shown to be distinct for neutrophils. Up regulation of CR3 on neutrophils was followed using an immunological technique along with election microscopy. The number of receptors was shown to up regulate with stimulation and clustering of the receptor was shown to occur upon stimulation (Malech, and Gallin). Studies have continued in this group on neutrophil heterogeneity and a systematic approach has been taken to distinguish neutrophil heterogeneity from differences in the state of neutrophil activation or neutrophil differentiation. Monoclonal antibodies that identify neutrophil heterogeneity have been identified and both developmental and activation antigens have been studied in a variety of disease states.

Important new developments occurred in studies of patients with chronic granulomatous disease. The bacterial disease group has the largest population of chronic granulomatous disease patients in the world and are learning highly important clinical facts about the care and treatment of these patients. They were one of the first groups to point out the heterogeneity of chronic granuloma disease patients. New molecular biological techniques have further delineated these differences between patient groups. Thus the X-linked form of the disease is associated with an absence of cytochrome B from the phagocyte and most patients with the autosomal form of the disease have been shown to possess cytochrome B. The group has demonstrated exceptions to this rule and even in some aberrant type of inheritance in one family. They have shown that interferon treatment of monocytes from CGD patients who have the cytochrome B gene may induce production of hydrogen peroxide. This may allow treatment of certain patients with chronic granulomatous disease which would be an important therapeutic advance. The group plans to test this therpay. The group has shown that steroid treatment to decrease granuloma size may provide an important adjunct to therapy even in infected individuals and in some cases may save the patient from surgery. An interesting concept being developed by the laboratory is that the products of the oxidative burst interact with various neutrophil products to inactivate them. One of the possible mechanisms of granuloma formation in these patients is that inflammatory factors which cause granulomas are formed by all neutrophils and inactivated by the oxidtive burst of normal cells. The inability to inactivate these products by CGD neutrophils leads to the formation of granulomas. Further studies by the group of a patient with specific granule deficiency have shown that these granules lack defensins, bactericidal proteins present in azurophil granules. Study of this patient who is quit unique and one of only a very few patients described with this defect is further allowing characterization of the normal physiology of neutrophil function (Gallin, Malech).

Yet another independent program of this group involves the study of endothelial cells in inflamation. One interesting finding of the group is that neutrophil independent promoters of vascular permeability such as histamine and platelet activating factor cause a cytosolic calcium burst in the endothelial cell, while neutrophil-dependent promoters of increased vascular permeability including C5a and Fmet-leu-phe and LTB4 elicit no change in endothelial cytosolic calcium. Another interest of the group has been in movement of chemoattractant factors, across an endothelial cell barrier. They show that endothelial cells bind formyl peptide chemoattractants and release them intact. They are currently pursuing the possibility that this interaction may lead to transport across the endothelial cell barrier.

Viral Disease Section

The Viral Disease Section has also had an active and successful year. One of the major research goals of this laboratory is to identify, map and characterize varicella-zoster viral genes, particularly during latent as opposed to productive infections. As part of this problem they wish to define the temporal sequence of gene expression and to determine the interaction of anti-viral drugs with viral-gene products through the molecular analysis of drug resistant mutants. The VZV genome has now been expressed in a recombinant library. Many genes from this library have now been mapped and the direction of transcripts determined. One particular early gene and its product have been looked at, the viral gene IE 175. This gene has been transfected into monkey cells and a stable cell line developed which expresses this protein. The expression of the gene and the protein have been studied in this cell line; this is leading to important information about control of gene expression. The completed sequence analysis of the thymidine kinase of 10 VZV strains has been accomplished, including six that are resistant to acyclovir by virtue of mutations in that gene. The nature and location of the genetic mutations have been determined. Included in the analysis is the first acyclovir resistent VZV strain recovered from a human.

After much effort a highly sensitive and specific in-situ hybridization probe has been developed and has successfully demonstrated RNA expression in the trigeminal ganglia of six of sixteen patients. Thus, the first steps at an understanding of a latent gene and its mechanism of reactivation have been taken. One interesting feature of these studies is the fact that VZV RNA signals are in a different set of cells than are the signals in HSV infection. This may be very important in determining the differences in clinical disease expression with these two viruses. As part of earlier studies on human enteral adenovirus a DNA probe which can be used to examine stool specimens was further examined in collaborative studies with the University of Manitoba. Its usefulness was demonstrated. Studies continue of the natural history and role of anti-viral therapy in mucocutaneous herpes simplex viral infection in normal and immune impaired patients. Long term studies of suppression with acycolvir of recurrent genital herpes have been completed. The fact that this drug is safe and well tolerated and does not lead to resistent mutants has emerged. Dosage schedules have been examined.

Endonuclease analysis of multiple viral isolates have shown that some individuals carry more than one strain of herpes simplex virus and can reactivate each of them independently. A method of causing reactivation of the human latent virus has been developed, which should allow further more specific studies of the mechanism of reactivation and anti-viral agents have been shown to block reactivation. A sensitive in-situ hybridization technique was developed after much effort which detects HSV in trigeminal ganglia. It was found that during latency viral specific RNA is complementary to the RNA encoding an important regulatory early protein ICPO. For example: Anti-sense transcripts have the capacity to inhibit the expression of the complementary normal (sense) messenger RNA. The presence of such a material might maintain the virus in a latent state. This may help explain the basis of latency and will be explored further (Straus).

The possible role of herpes virus as co-factors as in the progression and pathogenesis of AIDS is also being investigated. Recombinant clones containing the long terminal repeat sequences from HIV virus were linked to an indicator gene Expression of this virus was studied as a function of herpes virus infection of cells. It was found that herpes virus infection does stimulate the expression of HIV virus and the molecular biology of this effect is currently under investigation. Patients with HIV infection in the presence of other infections as occurs in the homosexual population appear to be at a much greater risk than patients who do not have other types of viral infection. The reasons for this are of interest and importance.

In a continuing major clinical project Dr. Straus and his group have been interested in sporadic neurasthenia associated with Epstein Barr and other viruses. The clinical and immunologic characteristics of a large group of patients with this chronic fatigue syndrome have been evaluated. A three year placebo controlled trial of acylovir in 27 patients with both chronic fatigue and unusual EBV serologies has been performed. The trial showed acylovir to be no better than placebo. Studies of other viral infections in this patient population are proceeding as well as are more sophisticated studies of EB viral latent disease in this patient group. Thus new herpes virusus are being looked for in this patient group as is the presence of latent viral genome using in-situ hybridization probes.

Mycotic Disease Section

The Mycotic Disease Section has continued its work with both cryptococcus and aspergillus as well as a number of other species of fungi during the past year. One project of the laboratory concerns lectin like recepters on murine pulmonary alveolar macrophages. These receptors bind to Aspergillus fumigatus conidia. Studies were performed of inhibition of these lectin like receptors by attachment of macrophages to glass slides coated with polysaccharides. The mannose-fucose receptor as well as the receptor for chitotriose appeared to be important in this binding. Interestingly a monoclonal antibody to a macrophage surface antigen F4/80 was found to be an inhibitor of binding. A new antifungal drug terbinafine (SSF86-327) is continuing to be studied and has promoise as an antifungal agent. Initial clinical studies of drug efficacy are now under way.

Aspergillus fumigatus has on its surface a component that acts as a complement inhibitor. It has now been shown to act as selective inhibitor of the alternative pathway. Inhibitory activity was shown to be unaffected by proteolysis and was abolished by periodate oxidation suggesting that it is a polysaccharide. Further column purification has been achieved and the component is near pure at the present time. This is of major importance because it may be possible to use it in patients as a complement inhibitor. These studies will be continued by Dr. Washburn at Bowman Gray School of Medicine. The group has also developed a rapid chemical technique for measuring flucytocine, an important therapeutic drug, which will be of major importance to those interested in control of drug levels in patients (Bennett).

Dr. Kwon-Chung's group as part of the clinical Mycology section has continued to study Cryptococcus neoformans with particular interest in the molecular biology and biochemistry of the organism. Differences between various subspecies of Cryptococus continue to be of interest to the group (for example urease activity inhibitable by EDTA was found present in one variety of Cryptococcus neoformans but not in the variety gattii). The matabolism of indole compounds was studied in these organisims and was simular to that found in melanoma cells with a single exception. During this year it was shown that torulopsus glabrata is a haploid organism while two Candida species which are closely related are diploid. A new fungual pathogen Cokeromyces recurvatus was found to cause cysititis in man.

Clinical Parasitology Section

We continue to work closely with the members of the Parasitology Group on projects of clinical interest. This material is included in more detail under the Parasitology program. Patients with parasitic diseases are being cared for on the wards of NIAID. In studies of these patients as well as in-field studies it has been possible to develop improved immunoassays for lymphatic filariasis, loiasis, onchocerciasis, strongyloidiasis, and ultrasonography was found to be beneficial in diagnosing schistosomal liver fibrosis. Therapeutic studies with Ivermectin have shown it to be an excellent drug for the treatment of Bancroftian filariasis. It is as good as the currently used drug diethylcarbamazine and has fewer side effects. It is useful against strongyloides but its curative effect has not been proven. Collaborative studies of a recombinant sporozoite vaccine for falciparum malaria in human volunteers has thus far been disappointing. The vaccine components will have to be reformulated in an attempt to induce a better immune response.

The Allergy Program

Allergic Disease Section

One major program of the Allergic Disease Section concerns the biochemical events which occurred during mast cell degranulation. A major study of mast cell morphology during mast cell degranulation has uncovered regional difference between skin, lung, and nasal mast cells. The group has become interested in neuropeptides. These peptides when injected into the skin can cause a wheel and flare response due to vascular leakage and are believed to be important in allergic phenomena, however the data is still largely inferential. Neurogenic stimuli of sensory nerves also leads to increased vascular permeability. In these studies it would appear that the mast cell is not involved, but the effect is directly or indirectly on the endothelial cell. A study is beginning, to examine the role of neuropeptide release in allergic phenomena. Thus far, receptors for these materials have been shown in human airways as well as other allergic sites. Neutrophils also are important in mediating the allergic response. In these studies a histamine releasing factor has been shown to be released from neutrophils which causes mast cells and basophils to release their mediators. One of the most easily controlled types of urticaria and angioedema occurs in the physical urticarias. For example some patients will develop angioedema or urticaria following cold exposure or exposure to vibration. New tests have been developed to explore this phenomena which allow the tiny amounts of histamine generated by such exposure to be measured reliably. The group in the past has shown that a certain small number of patients with idiopathic anaphylaxis appear to have allergic reactions to their own sex hormones. Some of these patients have been treated with LHRH analogue to modify this effect with success. This is being further studied now in separate "blind protocols". A rat leukemia model has been developed in the laboratory to help explore the complicated sequence of events that begin when IgE interacts with the IgE receptor on mast cells. This in turn aggregates the receptor, ultimately causing mediator release. In the first step a method was developed to permialize cells in such a way as to maintain their ability to degranulate when challenged with antigen. Thus it was possible to introduce various compounds into the cell but not destroy its integrity. The method being developed uses alpha toxin a protein secreted by Staph aureus. Onbinding to eucaryotic cells the protein polymerizes and forms a two to three nanometer pore which allows enterance of the substance to be tested. Thusfar successful permialization has been accomplished, a necessary first step in the project(Hohman).

The group continues its work on mucus like proteins. These are very important in the development of the asthmatic response, because the major pathologic feature of asthma is the secretion of large amounts of mucus which blocks the airways. Macrophages and mononuclear cells have already been shown by this group to secrete mucus secretogogues. Cortisone inhibits mucus secretion by lowering baseline effects and the generation of lipocortin is associated with the quantity of glyco-protein production. This year human neutrophils were added to the group of cells found to be able to secrete material that causes mucus to be elaborated by goblet cells. Endorphins also cause increased mucus secretion as do the neuropeptides. Study of pharmocologic control of this secretion is obviously the next step in the analysis of this problem.

Work continues on a attempt to make an IgE-ricin immunotoxin. Work thus far has allowed the production of a complex of the ricin a chain with the heavy chain of IgE. This complex binds to IgE receptors localizing the complex on the surface of mast cells as hoped. However internalization of the complex leading to destruction of the mast cells was not accomplished in previous studies. In later studies the IgE-ricin conjugates were injected interdermeally into rat's skin. Surprisingly this was effective in lowering intradermal histamine levels. Thus the possibility exists that in vivo these conjugates will be more effective than in vitro.

Another interesting area of investigation has involved canulating the nose and studying in a controlled and careful fashion nasal mucosa physiology. Studies have examined the production of mediators like histamine, immunoglobulins, mucus, and transudations of fluids from the circulation. In some cases production has simply reflected transudation of plasma from the circulation. With other stimulants production represents secretion by the cells of the mucosa. Methods were developed to differentiate these types of stimuli. Also drug studies were conducted. Hot spicy foods may cause rhinorrhea in sensitive subjects. This was found to involve an atropine-inhibitable muscarinic pathway. Histamine cause selective fluid secretion at low doses and increases vascular permeability and causes glandular secretion at high doses. Atropine prevented methacholine effects and reduced histamine induced grandular secretion. Thus it is possible to examine each of the components in a nasal response and determine the causative factors.

Mast Cell Physiology Section

One of the major interests of the Mast Cell Physiology Section is the culture of mast cells and the attempt to develop a line that can be used for study. Such lines derived from humans or primates are not available at the present time. In studies in this section non-primate bone marrow was cultured in the presence of mitogen stimulated spleen cell supernatants and was found to give rise to basophils. This suggested that in non-human primates the basophil is the culture equivalent of the rodent mast cell described by others. Human basophils were found to grow in mixed cultures using lectin stimulated peripheral blood mononuclear cell supernatants. It has been possible to keep these cells in culture for six to eight weeks and IL2 depletion of the culture supernatants facilitates growth; thus slowly the parameters of mast cell and basophil growth are being isolated and evaluated. A rodent model of mast cell mediated arthritis was developed during this year. This was a non-destructive transient arthritis induced by sensitization of knee joints with antigen specific IgE and then administration of antigen. The arthritis has been fully characterized. The ability to produce such an arthritis raises the possibility that mast cells may be involved in arthritis in man. Studies of rheumatoid synovial tissue has supported this interpretation in that mast cells are present and associated with lamina propria cells. The group found that human mast cells produce both heparin and chondroitin sulfate E. The latter molecule has never been identified in human mast cells.

The group also has had a major interest in patients with systemic mastocytosis and has the largest such patient population in the country. They are characterizing the disease and developing new methods of therapy. One interesting finding this year was that systemic mastocytosis patients have elevated levels of mast cell tryptase in their blood. This marker will be helpful in assessing disease activity. Children with mastocytosis were examined and in fact this disease was found to develop within the first six months of life. The presenting symptoms were similar to those in adults. Plasma histamine was generally found to be elevated in these patients and the children were found to be prone to develop peptic disease. Idiopathic anaphylaxis was separated from mastocytosis by biopsy and other evaluation.

Food allergy studies are also of interest to this group. Six normal subjects and nine patients with suspected sensitivity to aspartame were evaluated in a double blind challenge. Two of the patients with a history of aspartame sensitivity have positive skin tests to this drug. One individual with a positive history developed hives during a single blind challenge. But urine histamine levels remained normal in those cases. Hypersensitivity to aspartame was therefore quite infrequent and not readily diagnosed. Neither aspartame nor MSG was capable of provoking mast cell or basophil degranulation in vitro at nontoxic levels. In collaborative studies with Dr. Subba Rao in India the group showed a tRNA for the first time could be an allergen. In this case the tRNA was derived from shrimp to which the patient was allergic.

Mucosal Immunity Section

Dr. Strober's group has attempted to define at both the cellular and molecular level, the signalling events involved in immunoglobulin Ig heavy chain (C_H) class switching. In previous studies they showed that Con A-activated T cell clones derived from the murine Peyer's patch could induce sIgM+ B cells to differentiate into sIgaA+ B cell. During the last year period they extended this observation to humans by preparing PHA-activated T cell clones derived from human appendiceal cells. In these studies they found that appendiceal T cell clones could induce IgA synthesis in sIgM+,sIgA- and sIgM-,sIgA+ B cells but not sIgM+,A-B cells. Thus, switch T cells were present in human mucosal follicles but not peripheral blood. The capacity of various T cell-derived lymphokines, particularly IL-4 and IL-5, to act as switch or post-switch IgA B cell differentiation factors were also examined. In these studies populations of sIgM+,sIgA- and sIgM-, sIgA+populations from the murine Peyer's patch were prepared. IL-4 and IL5 containing supernatant derived from cultures of the EL-4 and D-10 cell lines supported IgA synthesis in LPS-stimulated sIgM-sIgA+B cells but not in sIgM-sIgA-B cell cultures. Recombinant IL-4 alone had little or no effect on IgA synthesis synthesis in any cell populations, whereas purified IL-5 had the same effect as the unpurified supernatant. These studies indicate that IL-5 acts as a post-switch IgA B cell differentiation factor. The effects of T cell-derived lymphokines of a B cell clone termed CH12.LX which is capable of undergoing terminal differentiation into an IgA-producing cell was also examined. CH12.LXB cells secreted large amounts of IgM and small amounts of IgA spontaneously in culture; LPS increased the IgA synthesis to some degree whereas IL-5 (purified) greatly increased IgA synthesis a while causing a decrease in IgM synthesis. Thus IL-5 can act either to preferentially increase proliferation of spontaneously arising IgA B cells or else act on pre-switched IgM B cells to increase their conversation to IgA cells.

As part of this group Dr. James has continued his studies of inflammatory bowel diseases. He has been particularly interested in the fact that T cells in human intestinal mucosa do not express one of the antigens, Leu-8, which is normally found on peripheral blood T cells. Cells with this marker and addition the CD4 marker are known to directly suppress immunoglobulin synthesis, while Leu-8 negative cells with the CD4 marker increase immunoglobulin synthesis. They found an antibody to Leu-8 enhanced the suppressive function of CD4 positive T cells. The cells of the lamina propria of the gut were isolated. At high T/B cell ratios it was found that intestinal

T cells provide more help for immunoglobulin synthesis than peripheral blood T cells. Treatment of cells with anti Leu-8 did not inhibit Ig synthesis. Of course gut cells have a low incidence of the marker. Similarly CD4 positive T cells were isolated and tested for suppressive function following treatment with anti-Leu-8. It was found that peripheral blood but not GI tract CD4 T cells suppress Ig synthesis after treatment. The overall thrust of the studies suggests that the diminished suppressor function of intestinal CD4 T cells is due to the diminished number of Leu-8 positive cells in this area rather than a major difference in their function. Presumably this difference is important in the pathogenesis of inflammatory bowel disease but the issue is still clearly under investigation. Using the marker for T cell activation, the IL2 receptor, this group investigated the state of activation of T lymphocytes in the intestinal lamina propria in comparison to circulating lymphocytes as well as cells isolated from the spleen and mesenteric lymph nodes in normal non human primates. Interestingly cells from gut have a significantly higher proliferation in response to IL2 when compared to other populations and they had a significantly higher percentage of IL2 positive cells as determined by a monoclonal anti-IL2 receptor antibody. Northern blot analysis with the cDNA clone specific for the IL2 receptor shows that unstimulated gut lymphocytes had easily detected messenger RNA for the receptor, whereas no message was detected in unstimulated lymphocyte population from other areas. The activation of this gene in the GI track lymphocytes was not associated with generalized activation as shown by other markers. Lymphocytes from this area when stimulated with a mitogen produce significantly more IL2 when compared with other populations. No message for IL2 was detected in these cells until stimulated but then was inducible with a lectin. Thus normal lamina propria lymphocytes make the receptor for IL2 in an unstimulated state but not IL2. In this respect they differ markedly from cells in the peripheral blood. How this fits in with the function of these cells in interacting with ingested antigens and in host defense is still under study. An understanding of normal pathophysiology is essential before abnormal function is understood.

Clinical Immunology Section

The Clinical immunology Section under Dr. Frank continues its studies of the role of complement and immune complexes in the production of immunologic damage. For many years this group has followed a group of patients who have the clinical signs of hereditary angioedema but who clearly have an acquired form of the disease. In the past the group noted that these individuals had a monoclonal antibody which recognized a limited number of determinants on the C1-esterase inhibitor molecule. This has been further evaluated in the past year. It has been shown that patients with this disease have a cleaved form of the C1-esterase inhibitor which has a molecular weight of approximately 94 kd. The function of the antibody which does not precipitate with the C1 inhibitor has been defined. C1 inhibitor is a serpine and operates by presenting a bait sequence to the enzyme to be inhibited. The enzyme cleaves the C1 inhibitor uncovering the new reactive group within the inhibitor molecule which forms a covalent bond with the substance to be inhibited. The antibody in these patients does not prevent the interaction of the C1 inhibitor with the substance to be inhibited. The enzyme to be inhibited cleaves the C1 inhibitor; however the antibody prevents the formation of a covalent bond. Thus the inhibitor continues to be cleaved but is functionally inactive and the patients develop a hereditary angioedema like picture. The NIH now has the largest group of such patients in the world and has followed one patient for along as 17 years.

Since the turn of the century the prozone phenomenon has been noted with a excess number of antibody molocules bound to a bacterial target leading to a fall off in lysis of bacteria. The step in the complement cascade in which this prozone resides has been analyzed and has been shown to involve the binding of C2 to C4 which falls off in plasma as the number of target bound C4 molecules increases. Current work is directed in determining whether this is due to some aspect of the chemical interaction between C2 and C4 or is due to the increased interaction of one of the regulatory proteins. In a potentially exciting development Dr. Hammer and colleagues have prepared a new method for the isolation of C2 which is simple and yields a molocule that is highly active. During development of this new method a 120 kd protein was discovered which appears not to have been previously described. It is present in large concentrations in plasma and appears to bind to C4. Further characterization of the structure and function of this protein is now underway.

In past years it was demonstrated that when IgG activates the complement cascade, C3 has a tendency to bind to the IgG molecule in the Fd portion. Once deposited on IgG it partially resists degradation by factors H and I although it does not resist degradation by CR1 + I. The biochemical consequences of this interaction have been under exploration for a number of years. It has been shown that C3b IgG heterodimer is much more active in hemolyzing bacteria and cells than is IgG alone, suggesting that the C3b can activate the alternative pathway when it resides on IgG in this protected site. In this site it can augment lysis. In the past year the binding of the heterodimer to monocytes and neutrophiles has been studied in detail. This molecule which forms naturally has important properties showing marked binding to both cell types even in the presence of serum concentrations of IgG. In addition it triggers phagocytosis much more efficiently than either molecule alone and a given number of C3b bound to IgG proves to be more efficient opsonin than the same numbers of C3b's on a cell with randomly deposited IgG in any number. Further studies of the opsonic potential of this important molecule are under way. In earlier studies with Dr. Gallins group, members of the Clinical Immunology Section showed that neutrophils from patients with chronic granulomatous disease have an increased level of phagocytosis and that normal granulocytes are down regulated during the phagocytic process. The consequence of this has been further explored by studying the neutrophil function of patients with various kinds of infections in collaboration with members of the staff at John Hopkins Hospital. It has been shown that neutrophils from patients with infection are upregulated to the extent that they do not show the normal down regulation of phagocytosis and absence of phagocytosis of minimally sensitized erythrocytes. On the other hand monocytes from patients with infection behaved in a perfectly normal fashion. The basis of these differences in phagocytosis is being further explored.

The function of complement receptors in endocytosis as well as in cell activation continues to be explored. Major emphasis this year has been placed on the C1q receptor and its interaction with cells. Unlike the C3 receptors the C1q receptor was shown in the past not be upregulated during cell activation. However interaction of C1q with its receptor was shown to augment IgG mediated phagocytosis enormously. This property of C1q was shown to reside in the head portion of the molecule rather than in the tail protion which binds IgG, indicating that it was receptor interaction that was responsible for this effect. This year these studies have been continued. It has been shown that the effect of C1q on phagocytosis is independent of the effect of phorbol ester on phagocytosis. C1q will remarkedly augment the effect of phorbol esters. This can lead to qualitative as well as quantitative difference between cell types. Thus in the presence of C1q monocytes which do not ingest complement coated particles in the presence of PMA can be induced to do so under the appropriate circumstances. To generalize the C1q affect, the role of C1q in augmenting the phagocytosis of cryptococci that were opsonized by IgG and or complement was studied. It was found that both phagocytosis and killing of cryptococci was markedly augmented following interaction with the Clq receptor. It was also shown that complement coated cryptococci are phagocytosed in the absence of IgG. Thus these cells are quite different from erythrocytes. The role of Clq receptor in the triggering of phagocytic cells and the activation of these cells will continue to be explored.

In the past years the basis of paroxysmal nocturnal hemoglobinuria, a rare hemolytic anemia, has started to be clarified. Earlier studies showed that PNH erythrocytes were missing, a complement regulatory protein, DAF, and work from this group showed that the precusors of PNH cells were not missing this regulatory protein. In collaboration with Dr. Muller-Eberhard at Scripps Clinic and Research Foundation the group showed this year that a second complement regulatory protein is completely missing from PNH cells. This protein Homologous Restriction Protein (HRP) serves to prevent the insertion of the membrane attack complex into cells to be lysed. It's absence in PNH erythrocytes explains the increased lytic suceptability of these cells and the lack of DAF explains the increase complement binding of these cells.

Studies of Herpes simplex virus type 1 have shown that this virus codes for the expression of receptors of complement binding protiens. In earlier studies Dr. Fries and his colleagues showed that one of the herpes glycoproteins, protein C, has C3b binding activity and is a potent modulator of complement mediated cytolysis, acting to cause decay of the alternative pathway on cellular activators. Recently the group has demonstrated that herpes simplex type 2 virus also induces expression of a C3 binding protein in infected cells. This protein has very different regulatory properties. It is already known that virus encoded proteins interact with components of the host defense process and are important in pathogenesis. The mechanisms by which these proteins alter the host defense process are still quite unclear and continued work in this area may allow further clarification of this point. An ongoing project explores the interaction of complement with another virus, parainfluenza virus. Work thus far has demonstrated that certain viral isolates will activate complement directly in the absence of antibody to deposit complement on the virus surface. Deposition does not lead to viral neutralization. The addition of antibody to this system does induce viral neutralization although the antibody alone is effective. In this system we hope to determine how antibody directs complement attack.

Dr. Joiner's group continues to study the interaction of complement with parasites and bacteria. In one interesting study with Neisseria gonorrhea it was found that purified components C1, C4, C2, and C3 bind to the bacterial surface in the absence of antibody. This is because the strain used bound and activated C1 directly. Addition of the normal plasma protein C1 inhibitor prevented this activation, Thus when the purified C1, C4, C2, and C3 and C1 inhibitor were added to the bacteria, no C3 was deposited on the bacterial surface. The addition of monoclonal antibody to the bacteria overcame the inhibitory effect of the C1 inhibitor and complement was again deposited. This in practical terms demonstrates the importance of last years finding that antibody partially inhibits the interaction of C1 with C1 inhibitor and antibody and C4 deposited together on a surface markedly block the interaction of C1 with the C1 inhibitor. Thus adventitious activation of C1 in plasma would be associated with marked inhibition of that C1 by C1 inhibitor. Directed activation of C1 via antibody in a complement cascade would not be easily inactivated by C1 inhibitor. Studies continue with both Trypanosoma cruzi and Leishmania major. Molecules have been identified on the metacyclic and tissue culture forms of the trypomastigotes of T. cruzi that inhibit the formation and accelerate the intrinsic decay of C3 convertase. The presence of these molecules explains the developmentally regulated resistance of these form to complement mediated lysis. These molecules have been purified and shown to migrate as 87 to 93 kd band. In separate studies the group has shown that C1q enhances infection of macrophages by trypomastigotes. Binding of complement fragments to Leishmania major was also examined. Nearly equivalent amounts of C3 were deposited on the noninfected serum sensitive parasites and on a subset of peanut agglutinin nonreactive, serum resistant and stationary phase parasites. Most of the C3 in both forms was present as C3b and was attached to a specific developmentally related glycolipid. The attachment of parasites to human mononuclear cells and mouse macrophages was inhibited by monoclonal antibodies to CR1. The mechanism of resistance of the L. major was release of the C5 through 9 complex from the organism surface, an event which is similar to that which occurs on certain types of bacteria. The earlier studies of this group that led to development of an ELISA assay for activation of the late components of complement by formation of a "neoantigen" continue. This assay was used to detect complement activation in a variety of autoimmune and rheumatologic diseases. Activation of C5b through 9 was the most sensitive indicator of complement activation and was elevated in both norocomplementemic and hypocomplementemic vasculitis of the Sjogren's type. Moreover, this material was found deposited in synovia in rheumatoid arthritis and in spinal fluid in patients with immunologic CNS disease in previous studies.

Awards and Honors

Two members of the LCI have been appointed Deputy Editors of the Journal of Immunology, Drs. Frank and Strober. There are six Deputy Editors of this prestigious Journal. Dr. Frank was asked to organize the complement section of the Federation of American Societies for Experimental Biology meeting for 1988. He was asked to participate with Drs. Frank Austin, Lawrence Lichtenstein and John Salvaggio in granting the Burroughs Wellcome Award for allergy research for $250,000 and the Hyde Watson Award of the American Academy of Allergy for $200,000. Dr. Frank was appointed Chairman of the Scientific Advisory Board of the Leonard Wood Memorial American Leprosy Foundation. Dr. Stephen Straus received an Outstanding Service Medal from the U.S. Public Health Service and Dr. John E. Bennet received the Commendation Medal of the Public Health Service. Dr. Stephen James received a Commendation Medal from the Public Health Service as well. Ms. Thelma Gaither received the NIAID Merit Award, the highest award given at the level of the Institute. Dr. Keith Joiner was elected to the American Society for Clinical Investigation. The Laboratory of Clinical Investigation now has seven members in that society. Drs. Metcalfe and Frank were asked to each write sections for the American College of Physicians' Review Course in Internal Medicine. Dr. Kwon-Chung was nominated as Distinguished Mycologist by the Mycological Society of America. Ms. Mary Lou Eury and Janet Dale each received Quality Increases because of the excellence of their work. Dr. Frank gave a named lectureship at Meharry Medical School and was visiting Professor at UCLA, Boston University and the New Jersey School of Medicine. He was a participant in the Swinford Conference of the University of Virginia and was invited to speak at the Pasteur Institute.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00043-22 LCI

PERIOD COVERED

Oct. 1, 1986 to Sept. 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunology and Chemotherapy of Systemic Mycoses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	J.E. Bennett,	Head, Clinical Mycology Section,	LCI, NIAID
Others:	R.G. Washburn	Medical Staff Fellow	LCI, NIAID
	V.L. Kan	Medical Staff Fellow	LCI, NIAID
	J.M. Delga	Visiting Fellow	LCI, NIAID
	C.H. Hammer	Senior Investigator	LCI, NIAID
	K.J. Kwon-Chung	Senior Investigator	LCI, NIAID
	W. Whelan	Guest Worker	LCI, NIAID

(See Attached Page)

COOPERATING UNITS *(if any)*

Clinical Pathology Dept., Clinical Center (Dr. Kroll)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. Lectin-like receptors on murine pulmonary alveolar macrophages which bind to Aspergillus fumigatus conidia in vitro were found to be inhibited by attaching macrophages to glass surfaces coated with saccharides. The most inhibitory saccharides were those which are also known to inhibit the mannose-fucose receptor and chitotriose, a component of chitin. In studies using fluid-phase inhibitors, the monoclonal antibody against a macrophage surface antigen, designated F4/80, was found to be an effective inhibitor of the conidia receptor on macrophages.
2. In studies of the new allylamine antifungal drug, terbinafine (SF 86-327), absorption after oral administration to mice was found to be greatly enhanced by dissolving the drug in 1% Tween and 5% DMSO. One patient was studied after 20 months of receiving this drug. Half-life of plasma clearance was strikingly slow, being 50 days.
3. Purification of the Aspergillus fumigatus complement inhibitor was facilitated by the discovery of anionic properties on ion exchange chromatography. In other studies on fungal opsonins, deposition of the third component of complement on Cryptococcus neoformans in normal human serum was much less in the variety gattii . This may help explain the poorer host defenses, as found in the slower response to chemotherapy among patients infected by this variety. Complement fragment C1q was found to enhance phagocytosis and killing of opsonized C. neoformans blastospores by human monocytes. It is feasible that this interaction may contribute to host defense against cryptococcosis.
4. Improvement in the rapid chemical assay of flucytosine in serum has been achieved using a deaminating enzyme. Ammonia release can be measured by automated techniques. Application for patenting this device has been made.

INVESTIGATORS CONTINUED:

C.M. Huang	Medical Staff Officer	LCI, NIAID
M. Kroll	Medical Staff Officer	LCI, NIAID
E. McManus	Medical Staff Fellow	LCI, NIAID
D.A. Bobak	Medical Staff Fellow	LCI, NIAID
M.M. Frank	Clinical Director	LCI, NIAID

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 AI 00045-19 LCI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on Interaction of Antibody and Complement on Production of Immune Damage

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Michael M. Frank, Chief, LCI/NIAID
Others: Dr. Louis Fries, Senior Staff, NIAID
Dr. Alejandro Malbran, Visiting Fellow, NIAID
Dr. Tom Lawley, Senior Staff Member, NCI
Dr. Kim Yancey, Senior Staff Member, Associate Professor of Medicine

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.7	2.2	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A new disease, C1 esterase inhibitor deficiency, associated with the formation of a monoclonal antibody to the C1 inhibitor was described. Seven patients have now been collected who are making this antibody of extremely restricted heterogeneity to one plasma protein. The antibody inactivates the plasma protein causing disease. Demonstration of this antibody has been difficult because of its low affinity and the fact that it does not precipitate with the antigen. Those difficulties have now been surmounted. The mechanism of action of the antibody has been studied. In its normal function C1 inhibitor presents a bait sequence to the enzyme to be inhibited and is cleaved. It then forms a covalent bond with the enzyme to be inhibited. The antibody does not appear to function to prevent cleavage of the C1 inhibitor but rather prevents the covalent bond from being formed when the C1 inhibitor is cleaved. In these patients the C1 inhibitor circulates as a fragment and has no functional activity. Methods of therapy are currently being developed.

C5a has been purified and C5a des arg are continuing to be studied for their effects in man. It has been shown that in human beings the wheel and flare response to C5a injections in the skin is attenuated if one studies patients with reduced polymorphonuclear neutrophils. These data are taken to indicate, in a preliminary way, that the C5a causes its effect on mast cells via a neutrophil effect; that is, it triggers neutrophils to release a factor which causes mast cells to degranulate. This is under further study at the present time.

As one increases the amount of antibody on the target it becomes impossible for complement to lyse the target. The basis for this phenomena, known since the turn of the century, has been examined. The step in which prozone occurs in the complement sequence has been identified and has to do with the binding of C2 to C4. The chemical reason why, as one increases the C4 concentration on the cell, C2 binding falls off is under investigation. The effect may have to do with the presence of regulatory proteins but this may not be the entire story.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00047-18 LCI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Clinical Studies of Patients with Known or Suspected Parasitic Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*
PI: E.A. Ottesen Head, Clinical Parasitology Section LCI/LPD, NIAID

Others: See Next page

COOPERATING UNITS *(if any)*

See next page

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Parasitology Section

INSTITUTE AND LOCATION
NIAID, NIH, BEthesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ ×(a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of these studies is to increase understanding of the pathogenesis of parasitic diseases and to improve their diagnostic, therapeutic and preventative measures. Investigations have been carried out in patients both at NIH and in the endemic areas of Guatemala (onchocerciasis), India (filariasis, kala azar), Mexico (leishmaniasis), and Sudan (schistosomiasis).

Diagnostic refinements have been made with immunoassays for lymphatic filariasis, loiasis, onchocerciasis and strongyloidiasis and with clinical measures (ultrasonography) for schistosomal liver fibrosis. Studies on pathogenesis have shown that differential immune responsiveness is correlated with lymphatic filariasis pathology (IgG3) and that local IgG antibodies and infiltrating T suppressor/cytotoxic cells are associated with the eye lesions of onchocerciasis. Antigen-specific immune unresponsiveness is characteristic of both kala azar and filarial infections, but the mechanisms underlying this immunologic unresponsiveness appear different.

Therapeutic studies with ivermectin have shown it to be at least as effective and safe at low dosages in bancroftian filariasis as the currently used drug diethylcarbamazine and to be the only drug demonstrated effective in mansonellosis. Against strongyloides its curative effect has been only temporary in some individuals.

Collaborative studies of a recombinant sporozoite vaccine for falciparum malaria in human volunteers has shown a protective effect in only one of six vaccinees. The lack of good booster responses suggests that modifications will be necessary for this prototype vaccine.

Other Professional Personnel
(name, title, laboratory, and institute affiliation)

T. E. Nash (Co-Principal Investigator)	Senior Investigator	LCI/LPD, NIAID
F.A. Neva (Co-Principal Investigator)	Senior Chief Investigator	LCI/LPD, NIAID
R. Hussain	Visiting Fellow	LPD, NIAID
A. Cheever	Assistant Chief	LPD, NIAID
L. Miller	Head, Malaria Section	LPD, NIAID
M. Lunde	Research Zoologist	LPD, NIAID
T. Nutman	Medical Staff Fellow	LPD, NIAID
R.G. Crystal	Chief	PB/NHLBI
D. Sacks	Staff Fellow	LPD, NIAID
P. Scott	Staff Fellow	LPD, NIAID
D. Freedman	Medical Staff Fellow	LCI, NIAID
E. Pearce	Visiting Fellow	LPD, NIAID
J. Ruthlein	Guest Researcher	LCI, NIAID
D. Ward	Medical Staff Fellow	LPD, NIAID
R. Davey	Medical Staff Fellow	LCI, NIAID
R. Lal	Visiting Fellow	LPD, NIAID
A. Aggarwal	Visiting Fellow	LPD, NIAID
S. James	Senior Investigator	LCI, NIAID
W. Rom	Visiting Associate	PB, NHLBI
C. Chan	Senior Staff Fellow	CB, NEI
R. Nussenblatt	Chief	CB, NEI
P. Brindley	Visiting Fellow	LPD, NIAID

Cooperating Units

University of Arizona (E. Petersen); Peace Corps Medical Office, Washington, DC (M. Mulligan); Department of Health, Guatemala (G. Zea-Flores); University of Michigan (J. Bennett); University of Khartoum, Sudan (M. Homeida); Harvard University, Boston, MA (S. Ackerman, F. Von Lichtenberg); Onchocerciasis Chemotherapeutic Research Center, Tamale, Ghana (K. Awadzi); Indian Council of Medical Research Tuberculosis Research Center, Madras, India (S.P. Tripathy, R. Prabhakar, P.R. Narayanan, V. Kumaraswami, R. Paranjape, and V. Vijayan); Centers for Disease Control (C. Reimer); Madras Medical College, Madras, India (K. Vijayasekaran); Institute of Dermatology, Santa Domingo, Dominican Republic (H. Bogaert); Instituto de Salubriadad y Enfermadades Tropicales, Mexico City, Mexico (O. Velasco); Rajendra Memorial Institute, Patna, India (A. B. Sen); Walter Reed Army Institute of Research, WAshington, DC (J. Chulay, W. Ballou, W. Hockmeyer, S. Hoffman, J. Berman, M. Grogl); SEMA, Inc., Rockville, MD (G. Phillips); Merck, Sharpe Dohme, Rahway, NJ (M. Aziz); and University of Maryland, Baltimore, MD (LM. Levine; Duke Univeristy S. Savarino); Pvt. Consult (B. Walton).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00048-17 LCI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Pathophysiology of Autoimmune Hemolytic Anemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Michael M. Frank, Chief, LCI/NIAID
Others: T. Quinn, Senior Investigator, LIR/NIAID
C. Hammer, Senior Investigator, LCI/NIAID
L. Fries, Medical Officer (Research), LCI/NIAID
M. Marques, Visiting Fellow, LCI/NIAID

COOPERATING UNITS *(if any)*

Dr. H.J. Muller-Eberhard, Scripps Clinic, La Jolla, CA

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.1	2.5	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Further studies of the biochemical basis of paroxysmal nocturnal hemoglobinuria (PNH) have been performed in collaboration with Dr. Hans Muller-Eberhard and the group at Scripps Clinic. Earlier work had shown that these erythrocytes are missing a complement regulatory protein, DAF, but that the precursors of PNH cells have this protein on their surface. Work this year has demonstrated that a second protein that regulates complement activation termed homologous restriction protein (HRP) is missing from PNH erythrocytes. Recently, this protein has been shown to prevent the insertion of the membrane attack complex formed by complement activation to the surface of cells. Thus the fact that this protein is missing explains the exquisite sensitivity of these cells to complement mediated lysis.

In vivo and in vitro studies of deposition of complement components on erythrocytes have been performed using both guinea pig and human materials. In the guinea pig it has been shown that there are marked differences in the numbers of components deposited by IgM and IgG antibody with IgM producing far more complement deposition on a guinea pig erythrocyte surface. The fragment of complement, also present on the surface, has been shown to be C3bi rather than C3b. In the human system but not in the guinea pig system, C3b was converted to C3d. In both systems, the C3 was shed rather rapidly from the red cell; an unexpected finding. These studies are being performed to determine what happens when red cells are injected into the circulation of human beings. Bacteria will undergo much the same processing, however, they are very difficult to study because their surfaces are complex and activate multiple pathways of complement activation. The importance of these studies is emphasized by the findings with Dr. Quinn that the clearance of complement coated red cells in patients with AIDS are distinctly abnormal, with the patients unable to remove the usual number of the cells from the circulation by phagocytosis once the binding reaction has occurred on the surface of the phagocyte. Thus, in the long run, discovering the events that take place during these pathophysiologic processes will help us understand the nature of the frequent infections that occur in this patient group.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00057-14 LCI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Basic Studies on Pathogenic Fungi

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboretory, and institute affiliation)*

PI:	K.J. Kwon-Chung	Research Microbiologist	LCI, NIAID
Other:	W.L. Whelan	Guest Worker	LCI, NIAID
	J.E. Bennett	Clinical Mycology Section Chief	LCI, NIAID

COOPERATING UNITS *(if any)*

LPD, NIAID

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Topics of current studies include 1. Urease activity of Cryptococcus neoformans; 2. Restriction fragment patterns of mitochondrial DNA from the two variets of C. neoformans; 3. melanogenesis in C. neoformans; 4. parasexual genetic studies of Candida and Torulopsis species; 5. DNA synthetic cycle of Candida species; and 6. Isolation of a new fungal pathogen.

The influence of EDTA on the urease activity of C. neoformans var neofrmans and C. neoformans var. gattii, was studied. Urease activity, one of the consistent diagnostic feature of the species, was inhibited by EDTA in the var. neoformans but not in the var. gattii. Nickel was found to be the only cation which eliminated the inhibitory effect of EDTA. This suggested that the urease of C. neoformans is a nickel assocated enzyme and that the mechanism of the enzyme synthesis is different in the two varieties although they cause the same disease.

The mitochondrial DNA from the four serotypes within the two varieties were digested with several endonucleases. The restriction pattern showed extensive polymorphism among the isolates of different serotypes indicating their different origin.Research was shown that phenoloxidase produced by Cr. neoformans oxidizes dihydroxyphenyl-alanine to dopaquinone after which melanin synthesis proceeds from dopaquinone via dopachrome and 5,6-dihydroxyindole, by auto-oxidation. Based on theseobservations, the pathway of melanogenesis in Cr neoformans was proposed to be the same as the classic Mason-Raper scheme found in melanoma cells with one exception, melanogenesis in Cr. neoformans is initiated form dopa and not from tyrosine. Parasexual genetic studies of Torulopsis glabrata, Candida parapsilosis, and Candida kruse revealed that Torulopsis glabrata is a hapoid organism while the two Candida species are not. Flow cytometric studies on three important species of Candida, C. albicans, C., tropicalis, and C. parapsilosis, showed that total DNA/cell of stationary phase cultures was markedly different between the species. The DNA content of C. parapsilosis and C. tropicalis were as high or higher than that of C. albicans. Histograms of Candida isolates in the exponential growth phase demonstrated that the majority of the population was in the G_2-M phase of the DNA synthetic cycle. The doubling time was fastest in the most virulent species. A new fungal pathogen, Cokeromyces recurvatus, was found to cause chronic cystitis in man.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00058-13 LCI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis and Chemotherapy of Herpes Virus Infections in Man

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S.E. Straus	Senior Investigator	LCI, NIAID
	J. Ostrove	Senior Staff Fellow	LCI, NIAID
	J. Felser	Medical Staff Fellow	LCI, NIAID
	K. Croen	Medical Staff Fellow	LCI, NIAID
	M. Sawyer	Medical Staff Fellow	LCI, NIAID
	A. Friefeld	Medical Staff Fellow	LCI, NIAID
	R. Holman	Medical Staff Fellow	LCI, NIAID
	J. Dale	Clinical Research Nurse,	LCI, NIAID

COOPERATING UNITS *(if any)*

J. Rooney, Senior Staff Fellow, LOM, NIDR; A. Notkins, Chief, LOM, NIDR; S. Nussinoff-Lehrman (Burroughs Wellcome Company)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goals of the project are to characterize the pathogenesis, the natural history, and the role of anti-viral therapy in mucocutaneous herpes simplex virus infections in normal and immune-impaired patients. Our clinical emphasis has been on genital herpes. We have completed analyses of four years of suppressive acyclovir treatments for patients with frequently recurring genital herpes. The drug has been found to be well-tolerated and not to induce drug resistant mutants at an appreciable rate. We determined that the most effective dosage schedule for suppressive acyclovir is dependent upon the inherent sensitivity of the individual's virus strain to acyclovir. Endonuclease analyses of multiple virus isolates show that some individuals carry more than one strain of herpes simplex virus and can reactivate each of those independently.

We have completed development of a human model by which herpes simplex virus infections are reactivated with ultraviolet light exposure to previously infected skin sights. A placebo-controlled acyclovir trial for blocking ultra-violet induced reactivation was initiated.

The major basic laboratory achievement over the last year has been the development of a highly sensitive in situ hybridization assay system by which herpes simplex virus specific messenger RNA can be detected in human trigeminal ganglia. We documented that during latency the virus-specific RNA is complementary to the RNA encoding an important regulatory immediate early protein ICP0. This finding may have enormous implications for understanding how herpes simplex latency is established and maintained in human tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00154-12 LCI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Events in Immediate Hypersensitivity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID
Others:	Martha V. White, M.D.	NRSA Fellow	LCI/NIAID
	Mary E. Revenis, M.D.	Guest Researcher	LCI/NIAID
	Marek L. Kowalski, M.D.	Fogarty Visiting Fellow	LCI/NIAID
	Paul T. McBride, M.D.	Medical Staff Fellow	LCI/NIAID
	Susan L. Wescott	Biologist	LCI/NIAID
	Diane Bradley	Chemist	LCI/NIAID

COOPERATING UNITS *(if any)*

Marc M. Friedman, Ph.D., Georgetown University (Contract# NO-1-A1-22665)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our analysis of events in immediate hypersensitivity have focused upon several major areas: 1) Analysis of mast cell morphology during degranulation showing regional differences between skin and either lung or nasal mast cells. 2) Mast cell secretagogues, particularly neurogenic or inflammatory derived. Sensory stimulation leads to release of neuropeptides and increased vascular permeability. This neurogenic inflammation is largely independent of mast cell activation but can involve mast cells if prolonged in duration. Conversely, neutrophils spontaneously release a factor known as histamine releasing factor (HRA-N) which causes mast cells and basophils to release mediators. This factor may be relevant to recruiting mast cells to participate in inflammatory reactions. 3) Mast cell mediators and their capacity to cause allergic disease. Histamine causes dramatic increases in lung cyclic GMP, localized to macrophages, epithelial and endothelial cells initially and followed by diffuse lung increases in cyclic GMP. Bronchial smooth muscle, however, is largely uninvolved. Histamine can be measured in venous plasma during delayed vibratory angioedema after cold urticaria and after routine allergy skin testing. 4) Modulation of allergic reactions. The capacity of LHRH analogue to modify progesterone-related anaphylaxis and the capacity of ketotifen to reduce exercise related anaphylaxis are being studied in separate blinded protocols.

These studies are helping to unravel the pathophysiologic events occurring during allergic diseases and will provide insights into improved therapy.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00155-12 LCI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Phagocytic Cell Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John I. Gallin, M.D.	Scientific Director, IRP,	NIAID
Others:	Harry L. Malech, M.D.	Head, Bacterial Dis. Sect.	LCI/NIAID
	Karen Lomax, M.D.	Senior Staff Fellow	LCI/NIAID
	Philip Murphy, M.D.	Medical Staff Fellow	LCI/NIAID
	Michael Klineberg, M.D, Ph.D	Med. Stf. Fel.	LCI/NIAID
	Hiroyuki Nunoi, M.D.	Visiting Associate	LCI/NIAID
	Joy Lennon, Ph.D	Visiting Associate	LCI/NIAID

COOP. UNITS: J Nath, Dept. Hematol., Walter Reed Army Inst. Res.,

COOPERATING UNITS *(if any)* Washington, DC; B Seligmann, Ciba Geigy, Summit, NJ; S Dreskin, A&R, NIADDKD; CJ White, IRP/NIAID; W Zimmerli, Kantonsspital, Basel, Switzerland; Y Ohno, Tenri Hosp., Japan; R Lerher, Dept. Medicine, Univ. California Los Angeles, Los Angeles, CA.

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Bacterial Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.1	3.4	1.7

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major objective of this project has been to study neutrophil function in health and disease. Determination of the biochemical and genetic basis of inherited diseases affecting phagocytes and associated with recurrent infections, and the diagnosis and treatment of these diseases is a major component of this project. Also within the scope of this project is the study of in vitro model systems of these inherited diseases using preparations of phagocytic cells derived from normal volunteers and patients. We have shown that Chronic Granulomatous Diseases (CGD) are a heterogeneous group of diseases resulting from absent H2O2 production by phagocytes, associated with recurrent infections. The X-linked form is usually associated with absence of cytochrome b from phagocytes, while the autosomal form does not lack cytochrome b. We have documented exceptions to this, and even an autosomal dominant form. We have found that Interferon gamma treatment of monocytes from some CGD patients (usually autosomal form) results in normalization of H2O2 production, suggesting that this may be a treatment modality. Granuloma formation in CGD may cause obstructions, but our studies indicate good outcome with steroid therapy. An in vitro model of granuloma formation using monocytes suggests that CGD patients may not effectively destroy inflammatory mediators such as LTB4, leading to exuberant cellular reaction and granuloma. We have been studying a patient with deficiency of specific granules. Further study indicated the absence of defensins, bactericidal proteins present in azurophil granules. Although this patient also fails to make lactoferrin, Southern blots indicate that he has the gene for this protein. This disease may be a defect in gene regulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00189-08 LCI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Clinical and Biochemical Studies of Human Enteral Adenovirus Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S.E. Straus	Senior Investigator, LCI, NIAID
OTHER:	J. Ostrove	Senior Staff Fellow, LCI, NIAID

COOPERATING UNITS (if any)

G. Hammond, University of Manitoba

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.2	.1	.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

Enteroadenoviruses (EAds) comprise a serogroup with two related types of adenoviruses which are associated with gastroenteritis in infants. We continued our studies of the epidemiology of enteric adenoviruses. During the past year we have concluded analyses of a highly sensitive and specific viral DNA probe for detecting EAd types 40 and 41 in human stools. Recent studies with investigators in Manitoba have verified earlier findings of the utility of this probe. During the coming year further molecular epidemiologic studies will be performed using this probe.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00192-09 LCI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on Immediate Hypersensitivity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Dean D. Metcalfe, M.D.	Head, Mast Cell Physiology Section	LCI/NIAID
Others:	Robert B. Bressler, M.D.	IPA Assignment	LCI/NIAID
	Brett V. Kettelhut, M.D.	Medical Staff Fellow	LCI/NIAID
	Arnold S. Kirshenbaum, M.D.	NRSA	LCI/NIAID

COOPERATING UNITS *(if any)*

Arthritis and Rheumatism Branch, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (R. Wilder), and The University of Washington (C.W. Henderson and S. Klebanoff)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mast Cell Physiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.00	2.25	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Non-human primate bone marrow, cultured in the presence of mitogen-stimulated spleen cell supernatants gives rise to basophils. This suggests that the non-human primate basophil is the culture equivalent of the rodent mucosal mast cell.

Human basophils of approximately 6% purity can be grown from human marrow using lectin-stimulated peripheral blood mononuclear cell supernatants. These cells are maximal in number at 2 weeks. Long-term cultures (6-8 weeks) give rise to small numbers of mast cells. IL-2 depletion of supernatants permits the growth of increased numbers of basophils.

Mast cells appear in rheumatoid synovial tissues in association with T-helper cells and are ablated by interarticular injection of steroids. A rodent model of a mast cell mediated arthritis has been developed. The resultant arthritis is non-destructive and transient. These observations, taken together, suggest that mast cells are involved in arthritis and may play a role in exacerbations of disease activity.

Primary microvascular endothelial cells produce both GM-CSF and a mast cell growth factor.

Human mast cells produce both heparin and chondroitin sulfate E. The latter molecule had heretofore never been identified in human mast cells. The biologic role of chondroitin sulfate E is unknown.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00249-06 LCI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Pathogenesis, Diagnosis, and Treatment of Systemic Mast Cell Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Dean D. Metcalfe, M.D.	Head, Mast Cell Physiology Section	LCI/NIAID
Others:	Robert B. Bressler, M.D.	IPA Assignment	LCI/NIAID
	Brett V. Kettelhut, M.D.	Medical Staff Fellow	LCI/NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mast Cell Physiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.00	1.00	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with systemic mastocytosis have elevated levels of mast cell tryptase in their blood. This mast cell marker will be useful in assessing disease activity. It is also present in the blood for several hours following anaphylaxis and should aid in the evaluation of patients experiencing unexplained syncopal episodes.

Gastrointestinal disease is common in patients with systemic mastocytosis. Fasting hyperhistaminemia is associated with gastric hypersecretion and peptic disease. Malabsorption, when present, was usually mild and associated with mast cell infiltrations in the lamina propria of the small intestine.

Pediatric mastocytosis was examined and found to usually present in the first 6 months of life. Presenting symptoms did not differentiate cutaneous from systemic forms. Plasma histamines are generally elevated, markedly so in diffuse cutaneous mastocytosis and in children prone to develop peptic disease.

Idiopathic anaphylaxis is not a form of "occult" mastocytosis, but rather a separate clinicopathologic entity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00250-06 LCI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical and Basic Studies on Inflammatory Diseases of the Gastrointestinal Tract

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Dean D. Metcalfe, M.D. Head, Mast Cell Physiology Section LCI/NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mast Cell Physiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.50	0.25	0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Six normal subjects and nine patients with suspected sensitivity to aspartame have participated in a NIAID-CRS approved blind challenge protocol. Two patients with a history of aspartame sensitivity had a positive skin test to aspartame. One individual with a history of adverse reactions developed several hives during single- but not double-blind challenge. Plasma histamine levels remained normal in all cases. Hypersensitivity to aspartame thus appears infrequent.

Two normal subjects, two asthmatics, and one individual with a history of hives from MSG have participated in a NIAID-CRS approved protocol for MSG sensitivity. No adverse responses were noted to MSG, and no changes occurred in plasma histamine levels. The "Chinese Restaurant Syndrome" was not observed.

Neither MSG nor aspartame was capable of provoking mast cell or basophil degranulation in vitro at nontoxic levels.

On a collaborative project with Dr. P.V. Subba Rao at the Indian Institute of Science, we have demonstrated IgE directed to shrimp-derived tRNA in the sera of two patients with shrimp allergy. This represents the first demonstration of tRNA as an allergen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00269-06
LCI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Neutrophil Subpopulations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John I. Gallin, M.D.	Scientific Director, IRP,	NIAID
Others:	Harry L. Malech, M.D.	Head, Bacterial Dis. Sect.	LCI/NIAID
	Christopher C. Brown, M.D.	Medical Staff Fellow	LCI/NIAID

COOPERATING UNITS *(if any)* R. Jacobson, Dept. Medicine, Georgetown Univ., Washington D.C.; B. Seligmann, Ciba-Geigy, Summit, NJ; P. Krause, Dept. Pediatrics, Hartford Hosp., Univ. Connecticut, Hartford, CT.; A. Segal, Hematology, Univ. Hospital, Univ. London, London, England.

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Bacterial Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	---

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Human neutrophil heterogeneity has been suggested on the basis of differences in chemotactic responsiveness, on the basis of results in our laboratory showing differences in Fc receptor mediated rosetting of IgG coated red cells, and more recently with monoclonal antibodies. Heterogeneity of binding of monoclonal antibodies to human neutrophils was examined using the fluorescent activated cell sorter. We developed an IgG monoclonal antibody, 31D8, that binds strongly to neutrophils that are activated with fMLP and more responsive chemotactically, representing about 95% of normal neutrophils. 31D8 antigen appears at the myelocyte stage of maturation. We used 31D8 to study patients with chronic myelogenous leukemia (CML), a clonal disease and showed that failure to express 31D8 on any mature appearing neutrophils correlated with progression of CML to blast crisis. In other studies we demonstrated that neonate cord blood neutrophils have a decreased number of 31D8 positive neutrophils which correlates with an increase in band forms. Similarly there is a marked decrease in the number of 31D8 cells after severe blunt trauma or after experimental infusion of endotoxin. Band forms and 31D8 weakly reactive cells comprise an overlapping but not identical population. 31D8 expression may be a measure of neutrophil maturation independent of nuclear morphology and may be useful to measure the degree of marrow release of neutrophils. Dr. Segal developed IgG monoclonal antibody, C10, which we have characterized as binding strongly to about 30 to 80% of neutrophils. Percent C10 positive neutrophils in the same individual is constant. Further studies are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00271-06 LCI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Purification and Characterization of Serum Complement Proteins and Fragments

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Carl H. Hammer, Senior Investigator, LCI/NIAID
Others: Michael M. Frank, Chief, LCI/NIAID
Ruth M. Jacobs, Medical Staff Fellow LCI/NIAID
Marisa B. Marques, Fogarty Fellow LCI/NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

(Study 1) We have developed a simplified protocol for the isolation and purification of human C2. C2 prepared by this method is homogeneous by biochemical, immunochemical and functional tests and is recovered with a specific C2 functional activity greater than that found in plasma. Interestingly, co-isolating with C2 by this procedure is a previously unidentified protein similar to C2 but with unique properties. This protein has no C2 hemolytic activity. It has a chain structure on reduced and non-reduced SDS -PAGE gels similar to C2 (MW 105,000) but is slightly heavier (MW 120,000). On agarose electrophoresis the 120 KD protein migrates anodal to C2 and elutes from DEAE Sephacel at slightly higher ionic strength implying amore acidic pI. Preliminary evidence suggests that this 120 KD protein binds to iC4/C4b and exhibits cofactor activity for enhancement of C2 lytic function. By a number of immunochemical techniques (Western blot, immunoelectrophoresis and double diffusion analysis), the 120 KD protein has been shown not to be antigenically related to factor B, C6, C7, C4BP or to any of eleven other serum proteins tested but may cross react with C2.

(Study 2) In order to study the molecular basis for the reported differences between the clearance of IgG and IgM-coated guinea pig red cells (EA), we purified guinea pig complement component C3, the principal protein of the complement cascade. We are investigating C3 binding to EA and the cleavage fragments remainng subsequent to incubation of sensitized cells in guinea pig serum containing ^{125}I-radiolabelled C3 as a means to study the C3 opsonins responsible for antibody mediated clearance. Purified IgG and IgM antibodies to guinea pig E cells were prepared from immune rabbit sera.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00272-06 LCI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Host Defense Against Pneumococcal Bacteremia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Michael M. Frank, Chief, LCI/NIAID
Others: David Bobak, Medical Staff Fellow, LCI/NIAID
Ronald Washburn, Medical Staff Fellow, LCI/NIAID
Andrea Tenner, Scientist One, Dept. of Biochem., Amer. Red Cross
Biomedical Research and Development, Rockville, Maryland

COOPERATING UNITS *(if any)*

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Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Although this project continues, we have not worked on phagocytosis of pneumococci this year because of the complexity of the organism. Pneumococci activate complement in a complex fashion, making it difficult to control the fragment deposited on the organism. Cryptococcus neoformans on the other hand requires opsonization for phagocytosis and is an encapsulated organism that is easily visualized and enumerated. Therefore, in extension of our studies on the C1q interaction with C1q receptors performed in Project 00447-02 we studied the ingestion and killing of the pathogenic fungus Cryptococcus neoformans (CN). The encapsulated blastospores of this organism resist phagocytosis by phagocytes. When coated with specific IgG or C3 fragments though, CN are ingested and killed by human peripheral blood phagocytes.

When opsonized with whole human serum, from which all antibody to Cryptococcus had been absorbed, we found deposition of complement on the surface of this organism. The complement coated organism could be phagocytosed proving that in the unactivated cell sufficient complement on the organism surface will allow phagocytosis in the absence of immunoglobulin. Studies were performed in which immunoglobulin receptors on the phagocytes were blocked. This did not block phagocytosis of the complement coated cryptococci. Thus the Cryptococcus is not like a red cell in that immunoglobulin is not required as a second signal to trigger phagocytosis. For the first time, it was found that triggering of the C1q receptor would markedly augment phagocytosis in killing of a microorganism, the Cryptococcus in this case. Phagocytosis and killing were augmented with both IgG and complement coated organisms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00275-06 LCI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Complement Receptor and C3 Mediated Opsonization

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Thelma Gaither, Research Biologist
Others: Michael M. Frank, M.D., Clinical Director
Hank Simms, M.D., Research Fellow
Thomas Quinn, M.D., Sr. Investigator
Leslie Gofney, Biologist
Divya Verma, Laboratory Worker

COOPERATING UNITS *(if any)*

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Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Polymorphonuclear neutrophils (PMN) and monocytes from patients with acute bacterial infections (inf. PMN/MON) and cells from normal healthy controls were examined in paired studies for phagocytosis of IgG coated sheep erythrocyte targets (EIgG and EAC3bIgG). Several cellular functions of PMN were followed in longitudinal studies of inf patients, including: binding of 125I-monoclonal antibodies to Fc and C3 receptors, superoxide and myeloperoxidase production, as well as phagocytosis. PMN of infected patients expressed hyperphagocytic activity when compared with normal PMN. Phagocytic activity was prominent in inf PMN in the presence and absence of sodium azide, which regulates oxidative byproducts. Unlike inf PMN, phagocytosis was minimal in control PMN and required azide for significant expression. Superoxide, but not myeloperoxidase, was reduced in inf PMN on several days of the study, suggesting that oxidative products may play a role in the enhanced phagocytic expression in inf PMN. The number of FcR and CR3 (receptors for IgG and iC3b respectively) were similar in controls and patients, whereas CR1 (C3b receptor) was elevated on PMN of several patients. Interestingly, phagocytosis by monocytes of infected patients was similar to that of normal monocytes, unlike PMN phagocytosis which greatly exceeded that of normal PMN controls. Thus, the "down regulation" of phagocytosis seen in normal PMN is reversed in cells of patients with infections and does not occur in monocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00276-06 LCI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of the Membrane Attack Complex of Human Complement

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K.A. Joiner	Senior Investigator	LCI, NIAID
Others:	M. Sanders	Medical Staff Fellow	Immunology Branch, NCI
	M. Frank	Clinical Director	LCI, NIAID

COOPERATING UNITS *(if any)*
Elaine Alexander and Thomas Provost, Johns Hopkins University, Baltimore, MD

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SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
LCI, NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies have continued on the demonstration of the terminal complement complex, C5b-9, in fluids and tissues of patient with immunologically-medicated disease. Serum immune complexes, serum complement levels and serum C5b-9 levels were measured in a group of Sjorgen's patients. Results in these assays were compared in patients with two distinct histopathologic types of inflammatory vascular disease, neutrophilic (hypocomplementemic) and mononuclear (hormocomplementic). SC5b-9 was the most sensitive indicator of complement activation and was elevated in both forms of vasculitis, indicating that complement activation occurs with both histopathologic types. Levels of C3, C4, and Ch_{50} were lower in patients with neutrophilic than with mononuclear vascular disease. These results further extend the utility of using C5b-9 as an absolute indicator of complement activation, in situations where levels of individual complement components are normal. The results indicate that complement activation may play a role in both histopathologic forms of vasculitis in Sjogen's syndrome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00277-06 LCI

PERIOD COVERED

September 30, 1986 to October 1, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism of Serum Resistance in Bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K. Joiner	Senior Investigator	LCI, NIAID
Others:	J.E. Schweinle,	IPA	LCI, NIAID
	C. Hammer	Senior Staff Fellow	LCI, NIAID
	M.M. Frank	Clinical Director	LCI, NIAID

COOPERATING UNITS *(if any)*

Penny Hitchcock, LMSF, RML, NIAID; Andrea Tenner, American Red Cross; Martin Blaser, M.D., VA Medical Center, Denver, CO.

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Clinical Immunology Section

INSTITUTE AND LOCATION

LCI, NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies were continued on the bactericidal activity of monoclonal antibodies directed against the H.8 proteolipid antigen of Neisseria gonorrhoeae. We found that levels of monoclonal which were bactericidal did not increase complement activation, exactly as we had previously reported with antibodies of different specificity. A new series of experiments was initiated testing the opsonic activity of the anti-H.8 monoclonal for human neutrophils, in conjunction with defined human complement fragments as opsonins. We used purified classical complement components to deposit defined complement fragments on the gonococcal surface. Of interest, strain JS3 activiated the classical pathway in the absence of antibody. Although activation was prevented by C1 inhibitor, addition of antibody was able to override the control of the inhibitor. Monoclonal anti-H.8 was maximally opsonic for JS3 in the presence of bound C3b, while C3b alone, anti-H.8 alone, or anti-H8 plus C4b were intermediate in effect. Therefore, levels of anti-H.8 which do not increase C3 deposition in serum are opsonic and bactericidal for strain JS3. These studies resolve disagreement in the literature about the complement pathway which is activated by Neisseria gonorrhoeae in serum, and rigorously address the antibody and complement requirements for opsonization of this important human pathogen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00278-06 LCI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of the Components of the Complement Cascade

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Carl H. Hammer, Senior Investigator, LCI/NIAID
Other: Michael M. Frank, Chief, LCI/NIAID
Ruth M. Jacobs, Medical Staff Fellow, LCI/NIAID
Marisa Marques, Visiting Fellow, LCI/NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have developed a simplified protocol for the isolation and purification of human C2. Interestingly, co-isolating with C2 by this procedure is a previously unidentified protein similar to C2 but with unique properties. This protein has no C2 hemolytic activity but may cross-react antigenically with C2. It has a chain structure on reduced and non-reduced SDS-PAGE gels similar to C2 (MW 105,000) but is slightly heavier (MW 120,000). On agarose electrophoresis the 120 KD protein migrates anodal to C2 and elutes from DEAE Sephacel at slightly higher ionic strength implying a more acidic pI. Similar iodine incorporation was seen with both the 120 KD protein and C2; ^{125}I-incorporation with N-chloro-benzenesulfonamide yielded 10X the specific radioactivity as with Bolton-Hunter reagent. The 120 KD protein is recovered in the same polyethylene glycol fraction as C2, binds to C4b/$_i$C4 in the presence or absence of C2 and is recovered in somewhat greater yield than C2, about 2mg/100ml EDTA plasma. Preliminary evidence suggests that this 120 KD protein binds to C4b/$_i$C4 and exhibits cofactor activity for enhancement of C2 lytic function. Between two and 10 fold potentiation of C2 lytic activity was observed by prior treatment of target EAC14b cells with up to 9 µg/ml 120 KD protein. By a number of immunochemical techniques (Western blot, immunoelectrophoresis and double diffusion analysis), the 120 KD protein has been shown not to be antigenically related to factor B, C6, C7, C4BP or to any of eleven other serum proteins tested.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00279-06 LCI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on Mucous Glycoproteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Michael A. Kaliner, M.D. Head, Allergic Diseases Section LCI/NIAID

COOPERATING UNITS *(if any)*

James H. Shelhamer, M.D., Carolea Logun, and Jens D. Lundgren, Critical Care Medicine, Clinical Center

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mucus secretion is a normal function of respiratory mucous membranes. Models for measurement of mucus production by cultured human and feline bronchial and nasal mucosae have been developed in order to examine the controls of mucus secretion. In addition to neurohormones and mediators of allergy, airways react to products generated by pulmonary macrophages and peripheral mononuclear cells with increased mucous glycoprotein secretion. The macrophage and mononuclear derived secretagogues are collectively being called macrophage/mononuclear cell derived mucus secretgogues (MMS).

Corticosteroids inhibit MGP release by lowering baseline secretion. Analysis of corticosteroid treated airways reveals a close correlation between lipocortin generation and MGP production.

Pulmonary inflammation with neutrophils is often associated with mucus production. Lysates of human neutrophils as well as supernatants from activated neutrophils cause airways to release MGP; this activity is not due to elastase, and identity of the mucus secretagogue is under study. Endorphins cause increased MGP secretion.

Substance P is found in close association with submucous glands. Substance P causes MGP secretion and the activity of substance P is markedly increased by protection from degradation by proteases with thiorphan.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00354 05 LCI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunoregulatory Defects in Inflammatory Bowel Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboretory, and institute affiliation)*

Stephen P. James, Investigator, Mucosal Immunity Section, LCI, NIAID
Warren Strober, Head, Mucosal Immunity Section, LCI, NIAID
Marjorie Kanof, NRSA Fellow, Mucosal Immunity Section, LCI, NIAID

COOPERATING UNITS *(if any)*
Claudio Fiocchi, M.D., The Cleveland Clinic Foundation, Cleveland Ohio

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Mucosal Immunity Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previous studies in our laboratory have shown that human intestinal mucosal T cells have little expression of the Leu-8 antigen which is normally found on the majority of peripheral blood T cells. In addition, CD4+, Leu-8+ T cells were shown to directly suppress immunoglobulin (Ig) synthesis, whereas CD4+, Leu-8 negative T cells provide help for immunoglobulin synthesis. It was also shown that modulation of the Leu-8 antigen with monoclonal anti-Leu-8 antibody enhances the suppressor function of CD4 T cells. To further examine the functional significance of decreased expression of the Leu-8 antigen on intestinal mucosal T cells, in the present study we compared the helper and suppressor function of lamina propria lymphocytes. At high T/B cell ratios, it was found that intestinal T cells provided more help for Ig synthesis than peripheral T cells. Whereas pretreatment of peripheral blood cells with anti-Leu-8 under crosslinking conditions significantly inhibited Ig synthesis, pretreatment of intestinal lymphocytes did not. In further studies of the mechanism of this phenomenon, CD4 T cells were isolated and tested for suppressor function following treatment with anti-Leu-8; it was found that peripheral blood, but not lamina propria CD4 T cells suppressed Ig synthesis. When highly purified populations of CD4+, Leu-8 negative and CD4+, Leu-8+ T cells were prepared from peripheral blood and intestinal lamina propria, it was found that the peripheral blood and intestinal T cell subpopulations had similar helper and suppressor function, respectively. Thus, the diminished suppressor function of intestinal CD4 T cells is due to the diminished number of CD4, Leu-8+ T cells in the lamina propria rather than any intrinsic difference in their function. This difference in T cell function in the intestine may be important for immunoglobulin production in the mucosa and in the understanding of intestinal inflammation in IBD.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00355 05 LCI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunoregulatory Defects in Primary Biliary Cirrhosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Stephen P. James, Investigator, Mucosal Immunity Section, LCI, NIAID

COOPERATING UNITS *(if any)*
E. Anthony Jones, Chief, Section on Liver Diseases, NIDDK

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Laboratory of Clinical Investigation

SECTION
Mucosal Immunity Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was not active during this fiscal year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00356-05 LCI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of the Regulation of IgA Immunoglobulin Synthesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Warren Strober M.D., Head, Mucosal Immunity Section, LCI, NIAID

Michael C. Sneller, M.D., Staff Associate, MIS, LCI, NIAID

Dennis Kunimoto, M.D., Fellow, Alberta Heritage Foundation, MIS, LCI, NIAID
Gregory R. Harriman, M.D., Staff Associate, MIS, LCI, NIAID

COOPERATING UNITS *(if any)*
Fred Mushinski, M.D., Senior Investigator, Laboratory of Genetics, NCI, NIH, Bethesda, MD 20892

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Mucosal Immunity Section

INSTITUTE AND LOCATION
Bethesda, MD 20892

TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 2.5	OTHER: .5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of this project is to define at both the cellular and molecular level, the signalling events involved in immunoglobulin Ig heavy chain (C_H) class switching. In previous studies we showed that Con A-activated T cells clones derived from the murine Peyer's patch could induce sIgM+ B cells to differentiate into sIgA+ B cells. During this period we extended this observation to humans by preparing PHA-activated T cell clones derived from human appendiceal cells. In these studies we showed that appendiceal T cell clones could induce IgA synthesis in sIgM+,sIgA- and sIgM-,sIgA+ B cells isolated by various techniques. In contrast, peripheral T cell clones could induce sIgM-,A+ B cells but not sIgM+,A- B cells. Thus, switch T cells were present in human mucosal follicles but not peripheral blood. In other studies we determined the capacity of various T cell-derived lymphokines, particularly IL-4 and IL-5, to act as switch or post-switch IgA B cell differentiation factors. In these studies we prepared populations of sIgM+, sIgA- and sIgM-, sIgA+ populations from the murine Peyer's patch and found that IL-4 and IL-5 containing supernatant derived from cultures of the EL-4 and D-10 T cell lines supported IgA synthesis in LPS-stimulated sIgM-sIgA+ B cells but not in sIgM+sIgA- B cell cultures. Recombinant IL-4 alone had little or no effect on IgA synthesis in any cell populations, whereas purified IL-5 had the same effect as the unpurified supernatant. These studies indicate that IL-5 acts as a post-switch IgA B cell differentiation factor. In a final series of studies we studied the effects of T cell-derived lymphokines of a B cell clone termed CH12.LX which is capable of undergoing terminal differentiation into an IgA-producing cell. CH12.LX B cells secreted large amounts of IgM and small amounts of IgA spontaneously in culture; LPS increased the IgA synthesis to some degree whereas IL-5 (purified) greatly increased IgA synthesis a while causing a decrease in IgM synthesis. Thus IL-5 can act either to preferentially increase proliferation of spontaneously arising IgA B cells or else act on pre-switched IgM B cells to increase their conversion to IgA cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 00357-05 LCI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of the Autologous Mixed Lymphocyte Reaction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Warren Strober, M.D. Chief, Mucosal Immunity Section, LCI/NIAID

Other: Stephen James, M.D. Investigator, Mucosal Immunity Section, LCI/NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been inactive during the current period.

LID

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00396-03 LCI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Complement Receptors: Regulation of Expression and Cell Biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*
PI: Alejandro Malbran, Visiting Fellow, LCI/NIAID
Others: Louis Fries, Senior Staff Fellow, LCI/NIAID
Michael M. Frank, Chief, LCI/NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

This project has been included in number 00397 and should be terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00397-04 LCI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Interactions of C3b with Immunoglobulin G-Regulation of C3b Function by Antibody

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Louis F. Fries, Medical Officer (Research), LCI/NIAID
Others: Alejandro Malbran, Visiting Fellow, LCI/NIAID
David A. Bobak, Medical Staff Fellow, LCI/NIAID
Michael M. Frank, Chief, LCI/NIAID
John Zurlo, Medical Staff Fellow, LCI/NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Immunoglobulin G is known to enhance the rate and extent of alternative complement pathway activation on a variety of targets, and to facilitate complement-mediated destruction of some bacteria and virus-infected cells. We have shown that C3b residues covalently deposited on IgG are relatively protected from the actions of serum factors H and I, and hence have enhanced capacity to sustain alternative pathway activation. Recent studies have been directed at the interaction of covalent C3b-IgG complexes with phagocytic cells. Such heterodimeric complexes have been shown to interact readily with human polymorphonuclear leukocyte CR1 and Fc receptors under conditions which mimic the plasma environment and preclude binding of the individual proteins (C3b and IgG). Heterodimer binding has been shown to be dependent on both complement and immunoglobulin receptors and to result in a physiologic response by the cell: ingestion of the bound complexes. In additional studies, we have shown that C3b-IgG heterodimeric complexes are highly efficient opsonins and elicit greater phagocytic activity by both polymorphonuclear leukocytes and mononuclear phagocytes than opsonization of target particles by equal numbers of C3b and IgG residues randomly deposited.

Parallel studies have been undertaken to characterize the effect of CR1 crosslinking on phagocyte function. We have shown that CR1 crosslinking elicits receptor internalization, with some degree of subsequent recycling, and in addition primes at least mononuclear phagocytes for enhanced IgG-dependent phagocytosis.

Future directions will include: 1) studies of mechanisms of transmembrane signal generation by C3b-IgG complexes, 2) further exploration of phagocyte "priming" for various host defense functions and 3) potential pathogenetic consequences of C3b-IgG interactions in disease states.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00398-04 LCI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on the Interaction of Complement and Parasites

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	K. Joiner	Senior Investigator	LCI, NIAID
Others:	S. Puentes	Medical Staff Fellow	LCI, NIAID
	M.T. Rimoldi	Fogarty Fellow	LCI, NIAID
	S. Fuhrman	Medical Staff Fellow	LCI, NIAID
	B.F. Hall	Medical Staff Fellow	LCI, NIAID
	D. Bobak	Medical Staff Fellow	LCI, NIAID
	C. Hammer	Senior Staff Fellow	LCI, NIAID

Others--listing continued on page 5.

COOPERATING UNITS *(if any)*

Andrea Tenner, American Red Cross

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
LCI, NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	4.3	.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Experiments were continued examining the interaction of complement with Trypanosoma cruzi and Leishmania major, in an effort to elucidate the mechanism of parasite preadaptation to complement resistance, and to define the parasite and host molecules involved in invasion of host cells.

We identified molecules produced by metacyclic and tissue culture trypomastigotes of T. cruzi which inhibit the formation and accelerate the intrinsic decay of complement C3 convertases and hence explain the developmentally regulated resistance of these forms to complement-mediated lysis. These molecules, which are protease sensitive and retained on Concavalin A. sepharose, were purified by FPLC anion exchange and chromatofocusing from ^{35}S-labeled parasites, and migrated as a 87-93 kd band by SDS-PAGE. In separate studies, we have shown that C1q enhances infection of macrophages by trypomastigotes.

The deposition of complement on infective and non-infective promastigotes of Leishmania major was compared. Nearly equivalent amounts of C3 were deposited on non-infective, serum sensitive, log phase parasites and on a subset of peanut-non-agglutinable (PNA$^-$), serum resistant, stationary phase parasites. Almost 85% of C3 on both log and PNA$^-$ was present as C3b, which was attached to the developmentally regulated glycolipid, excreted factor a critical component for parasite infectivity. The mechanism of serum resistance in PNA$^-$ was different from that in T. cruzi trypomastigotes since in L. major, a C5b-9 complex formed but was released from the parasite surface without causing a lethal event. The attachment and entry of parasites into human mononuclear cells and mouse macrophages was inhibited by monoclonal antibodies to the C3b receptor, CR1.

Complement deposition on serum resistant tachyzoites of Toxoplasma gondii was tested. The form of C3 on the parasite surface and tachyzoite molecules binding C3 were determined, as a prelude to examining the role of C3 fragments and antibody in cell invasion by T. gondii.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00428-03 LCI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
IgE Immunotoxins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID
Others:	Howard Boltansky, M.D.	Senior Staff Fellow	LCI/NIAID

COOPERATING UNITS *(if any)*
Richard Youle, Ph.D., National Institute of Neurological and Communicative Disorders and Stroke; and Otto Gansow, Ph.D., National Cancer Institute

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Allergic Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mast cells are the cellular nidus of allergic diseases and the cell responsible for disease in urticaria pigmentosa and systemic mastocytosis. This project is designed to ablate mast cells by attaching cytotoxic agents to IgE or antigen and selectively introducing the toxic product into mast cells. IgE linked to ricin kills RBL cells in vitro spontaneously or after cross-linking the IgE with anti-IgE. IgE linked to ricin's A chain kills in the presence of monensin, a carboxylic ionophore, or in the absence of monensin at higher concentrations. IgE-ricin A conjugates injected intradermally significantly lower rat cutaneous histamine levels in vivo suggesting that this agent might work in vivo as well.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00429-03 LCI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on Nasal Responses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID
Others:	Gordon D. Raphael, M.D.	Medical Staff Fellow	LCI/NIAID
	Aleksander Z. Gawin, M.D.	Special Volunteer	LCI/NIAID

COOPERATING UNITS *(if any)*
Robert F. Bonner, M.D., Biomedical Engineering and Instrumentation Branch, Division of Research Services; and James H. Shelhamer, M.D., Critical Care Medicine, Clinical Center

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Allergic Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Allergic Diseases Section began studying nasal provocation testing in 1982 when Dr. Howard Druce joined the section. Over the next 3 years, the laboratory focused on studies of nasal physiology with the plan to eventually study the allergic response in the nose. Dr. Robert Bonner developed the technique of laser-Doppler velocimetry (LDV) and, with Dr. Druce, adapted this technology to the study of nasal mucosal physiology. Using parameters including blood volume, blood flow, and pulsatility, baseline LDV measurements were recorded after saline nasal provocation, and then after provocation with methacholine, histamine, atropine, lidocaine, phenylephrine, and oxymetazoline.

Nasal lavages were collected after nasal provocation, and these samples were assayed for total protein and albumin as a crude estimate of nasal secretion, histamine, and IgA.

Methacholine was found to stimulate protein secretion without a relative increase in albumin, indicating selective glandular secretion. Histamine stimulated a selective albumin secretion (indicating increased vascular permeability) at low doses and both glandular secretion and vascular permeability at high doses. Atropine prevented methacholine effects and reduced histamine induced glandular secretion. Therefore, we can analyze the components of nasal protein secretion and determine their source.

Hot, spicy foods may cause rhinorrhea in sensitive subjects. This gustatory rhinitis was found to involve an atropine-inhibitable muscarinic pathway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00430-03 LCI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of Varicella-Zoster Virus Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S.E. Straus	Senior Investigator	LCI, NIAID
OTHER:	J. Ostrove	Senior Staff Fellow	LCI, NIAID
	J. Felser	Medical Staff Fellow	LCI, NIAID
	K. Croen	Medical Staff Fellow	LCI, NIAID
	M. Sawyer	Medical Staff Fellow	LCI, NIAID
	A. Freifeld	Medical Staff Fellow	LCI, NIAID
	G. Inchauspe	Visiting Fellow, LCI, NIAID	

COOPERATING UNITS *(if any)*

J. Hay (USUHS), W. Ruyechan (USUHS)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ x(a) Human subjects ☐ (b) Human x tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major basic research focus of this laboratory involves this project, with the following goals:

1) to identify, map and characterize varicella-zoster virus genes and proteins active in latent or productive infections.

2) to define the temporal sequence of gene expression.

3) to determine the interaction of antiviral drugs with viral gene products through a molecular analysis of drug-resistant mutants.

To accomplish these ends we have constructed a variety of recombinant libraries of the complete VZV genome. During the past year we have concluded studies of the finer mapping and directionality of viral transcripts. We mapped the viral IE 175 immediate early gene. We have tranfected that gene into monkey cells and developed cell lines which stably express it. In the cell lines the expression of the gene appears to be autoregulated. Using assays by which we could measure the activity of viral gene promoters we detected a number of VZV genes that are transactivating and transactivatible. During the past year we have also completed sequence analysis of the thymidine kinase locus of ten VZV strains, including six that are resistant to acyclovir by virtue of mutations in that gene. We identified the nature and location of base mutations which render this gene product nonfunctional. Included in the analyses is the first acyclovir-resistant VZV strain recovered from a human. During the past year we have successfully established a highly sensitive and specific in situ hybridization system using ^{35}S labelled synthetic RNA probes. Such probes VZV RNA expression in the trigeminal ganglia of six of 16 subjects. Continuing efforts will be directed at better mapping and characterizing the RNA expressed during VZV latency.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00432 03 LCI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Regulation of Mucosal Immune Responses in Non-human primates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

Stephen P. James, Investigator, Mucosal Immunity Section, LCI, NIAID
Martin Zeitz, Guest Researcher, Mucosal Immunity Section, LCI, NIAID

COOPERATING UNITS *(if any)*

Warner Greene, M.D., Metabolism Branch, NCI

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Mucosal Immunity Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS.	PROFESSIONAL:	OTHER
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Expression of interleukin-2 receptors (IL-2R) and synthesis of interleukin-2 (IL-2) play central roles in T cell activation, proliferation and differentiation. This study investigated the state of activation of T lymphocytes in the intestinal lamina propria in comparison to circulating lymphocytes and lymphocytes isolated from the spleen or mesenteric lymph nodes in normal non-human primates. Although mitogen-induced proliferation was similar in the different lymphocyte populations, lamina propria lymphocytes had a significantly higher proliferation in response to recombinant IL-2 compared to the other populations. In agreement with this finding, lamina propria lymphocytes had a significantly higher percentage of IL-2R positive cells as determined by staining with fluoresceinated monoclonal anti-IL-2R antibody, whereas very few IL-2R positive cells were detected in peripheral blood, spleen and mesenteric lymph node lymphocytes. Two color immunofluorescence staining showed that both CD4+ and CD8+ lamina propria T cells were IL-2R positive. It was also found that the percentage of MHC class II positive T cells was higher in the lamina propria. Northern blot analysis with a cDNA specific for the IL-2R showed that unstimulated lamina propria lymphocytes had easily detectable IL-2R mRNA, whereas no IL-2R mRNA was detected in unstimulated lymphocyte populations from the other sites. The activation of the IL-2R gene in lamina propria lymphocytes was not associated with the activation of other cellular genes (actin, MHC class I). Although no IL-2 bioactivity was measured in culture supernatants of unstimulated lymphocytes, ConA-stimulated lamina propria lymphocytes produced significantly more IL-2 compared to the other populations. This finding was confirmed at the molecular level since no IL-2 mRNA was detectable in unstimulated lamina propria lymphocytes but was inducible by ConA stimulation. Thus, normal lamina propria lymphocytes have a selective expression of some, but not all genes associated with T cell activation, indicating that T cells in this site are in a unique state of activation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00447-03 LCI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

C1q: Its Biosynthesis and Biological Functions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Michael M. Frank, Chief, LCI/NIAID
Others: David Bobak, Medical Staff Fellow, LCI/NIAID

COOPERATING UNITS *(if any)*

Andrea Tenner, Scientist I, Dept. of Biochemistry, American Red Cross Biomedical Research and Development, Rockville, MD

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Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goals of this project are twofold: 1) the characterization of C1q receptor (C1qR) expression on human polymorphonuclear (PMN) and mononuclear phagocytes, and 2) investigation into the modulation by C1q of FcR- and complement receptor-mediated phagocytosis by monocytes and macrophages.

Elutriated monocytes were cultured for seven days in Teflon flasks ("macrophages") containing HL1 (a serum-free medium) or RPMI with 12% fetal bovine serum (FBS). Macrophages were adhered to human serum albumin (HSA)- or C1q-coated LabTek chamber slides, and the ingestion of sheep erythrocytes opsonized with IgM and C4b(EAC4b) was measured. EAC4b are bound to and are ingested via CR1. HL1-cultured macrophages did not ingest EAC4b whether the macrophages were adherent to HSA or C1q. RPMI/12% FBS-cultured, HSA-adherent macrophages also did not ingest EAC4b; however, C1q adherent macrophages did phagocytose EAC4b. This suggested that C1q, in the presence of other factors, could activate CR1 for ingestion. Because tumor promoting phorbol esters, such as phorbol dibutyrate (PDBu). activate CR1-mediated phagocytosis by macrophages, we investigated the effects of C1q on PDBu-stimulated phagocytosis in HL1-cultured macrophages. In the presence of PDBu, C1q-adherent macrophages markedly increased the ingestion of EAC4b over that level measured for macrophages treated with PDBu alone. The concentration of PDBu and time of stimulation were important determinants of maximal synergy with C1q. This C1q-stimulation of monocytes was likely mediated via C1qR, because the effect was reproduced by purified C1q tail fragments and blocked by $F(ab')_2$ anti-C1q. Furthermore, freshly isolated monocytes, which have not been reported to ingest via CR1 even when stimulated with PDBu, became competent to phagocytose EAC4b when stimulated concurrently by PDBu and C1q.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00469-02 LCI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Complement: Studies in Viral Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Louis F. Fries, Medical Officer (Research), LCI/NIAID
Others: Srikantan Vasantha, Visiting Fellow, LCI/NIAID
Brian Murphy, Senior Investigator LID/NIAID

COOPERATING UNITS *(if any)*
Harvey M. Friedman, University of Pennsylvania; Gary H. Cohen,University of Pennsylvania; Roselyn J. Eisenberg, University of Pennsylvania

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.5	1.25	0.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Infection with herpes simplex type 1 (HSV-1) induces expression of receptors for C3b on mammalian cells previously devoid of such receptors. We have shown that the isolated glycoprotein responsible for this activity (gC) is a potent modulator of complement-mediated cytolysis in-vitro, acting by at least two mechanisms. Recently, we have demonstrated that herpes simplex type 2 (HSV-2) also induces expression of a C3b binding protein which corresponds to HSV-1 gC, but has different regulatory properties vis-a-vis the complement cascade. We have demonstrated that HSV-1 gC cannot support degradation of C3b, but does enhance the activity of the normal plasma C3b regulatory proteins. HSV-1 gC does not appear to fundamentally alter the interaction of human cellular C3b receptors (CR1) or iC3b receptors (CR3) with fluid phase or target-bound C3b or iC3b. Investigations in this area are ongoing.

Studies have been initiated to examine the interactions of complement and antibody in neutralization of para-influenza virus. We have found that para-influenza virus 3 activates complement via the classical pathway in the absence of antibody. This activation is inocuous to the virus unless antibody is present, in which case neutralization and frank viral disruption occur. The antibody effect appears independent of the overall rate or extent of C3 deposition, and can be readily shown with monoclonal antibodies lacking intrinsic neutralizing capacity. Further studies will be directed at elucidating the mechanism of this IgG-complement interplay in viral neutralization.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00470-02 LCI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Sporadic Neurasthenia Associated with Epstein-Barr Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S.E. Straus	Senior Investigator, LCI, NIAID
OTHER:	J. Dale	Clinical Research Nurse, LCI, NIAID
	J. Ostrove	Senior Staff Fellow, LCI, NIAID
	K. Croen	Medical Staff Fellow, LCI, NIAID
	Holman	

COOPERATING UNITS *(if any)*

M. Kruesi (LCS, NIMH), G. Tosato (BOB, FDA), G. Armstrong (BOB, FDA), W. Henle (Children's Hosp., Philadelphia), M. Dalakas and J. Sever (NINCDS)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1.75	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to characterize unusual chronic infections with Epstein-Barr virus and to ascertain whether Epstein-Barr virus or other infectious agents can initiate a syndrome of chronic fatigue. We identified and are further exploring a series of clinical and immunologic abnormalities in nearly 140 patients to date. We completed a three year placebo controlled trial of acylovir therapy in 27 patients with the chronic fatigue syndrome and unusual EBV serologic profiles. The trial showed acylovir to be no better than placebo. During the past year we heightened our focus on individuals whose illness began with well defined acute infections particularly documented acute infectious mononucleosis. We further explored the prevalence of antibodies to individual EBNA proteins in both normal and fatigued populations. We have initiated detailed neurologic, neuroendocrologic, and neuropsychologic studies in selected subjects with this syndrome. We also initiated molecular studies of the virus carried by patients with severe chronic Epstein-Barr virus infection and with the chronic fatigue syndrome. To this end we have developed an in-situ hybridization system by which we detect EBV gene expression at increased rates in the tissues of some patients with chronic EBV infections.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00481-02 LCI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)
Receptors and Transduction Mechanisms in Human Phagocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Harry L. Malech, M.D.	Head, Bacterial Dis. Sect.	LCI/NIAID
Others:	John I. Gallin, M.D.	Scientific Director, IRP,	NIAID
	Karen Lomax, M.D.	Senior Staff Fellow	LCI/NIAID
	Philip Murphy, M.D.	Medical Staff Fellow	LCI/NIAID
	Daniel Rotrosen, M.D.	Medical Staff Fellow	LCI/NIAID
	Judith Falloon, M.D.	Med. Staff Fel. (former)	LCI/NIAID

Coop. Units: Allen Speigel, MDB, NIADDKD; Marc Friedman, Molec. Virol. Immunol. Div., Georgetown Univ., Rockville, MD;

COOPERATING UNITS (if any) Edward Benz & Mary Todd, Dept. Medicine, Yale Univ., New Haven, CT.; Donald Heiman, Dept. Medicine, Chicago Med. School, North Chicago, IL.; Richard Sullivan, Dept. Medicine, Boston Univ. Boston, MA.

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Bacterial Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.8	1.8	1.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

Human neutrophils and monocytes are attracted to sites of inflammation and engulf invasive microorganisms or host cell debris. These phagocyte responses are mediated by surface receptors for a variety of substances released by infective agents or host cells, or derived from host plasma. The purpose of this project is to define the biochemical structure and transduction mechanisms of some of these surface receptors. More specifically, the present studies focus upon: (i) the receptor for bacterially derived formyl peptides, the formyl peptide chemotactic receptor (FPCR); (ii) pertussis toxin inhibitable GTP binding regulatory proteins (Gi) in myeloid cells required for FPCR and other receptor mediated cellular responses; (iii) the iC3b (CR3) receptor and other surface antigens on neutrophils. Using affinity labelling, we showed that FPCR contains both complex- and high mannose-type N-linked polysaccharides. We showed that FPCR appears at the late promyelocyte stage of myeloid differentiation accompanied by functional responses to formyl peptide. Using antibodies and oligonucleotide probes to detect protein and mRNA of different alpha subunits of GTP regulatory proteins, we showed that myeloid cells contain at least two subtypes of Gi and do not contain the Gi subtype most common in brain tissue. The presence of distinct subtypes of Gi may be important for neutrophil functions mediated by different receptors. With immunogold-electron microscopy we showed that the density of neutrophil surface CR3 doubles after stimulation with phorbol myristate acetate and that CR3 are clustered on the cell surface. This arrangement may be important for the neutrophil adherence mediated by this receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00482-02 LCI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Endothelial Cells in Inflammation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Daniel Rotrosen, M.D.	Medical Staff Fellow	LCI/NIAID
Others:	John I. Gallin, M.D.	Scientific Director, IRP,	NIAID
	Harry L. Malech, M.D.	Head, Bacterial Dis. Sect.	LCI/NIAID
	Philip Murphy, M.D.	Medical Staff Fellow	LCI/NIAID

COOPERATING UNITS *(if any)*
Kevin Foskett, Ph.D., Department of Physiology, Armed Forces Radiobiology Research Institute, Bethesda, MD

LAB/BRANCH Laboratory of Clinical Investigation

SECTION Bacterial Diseases Section

INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	---

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The vascular endothelial cell is uniquely situated to play an active role in the transduction of pro-inflammatory signals from the interstitial tissues to circulating neutrophils. The goals of our principal projects are (i) to study the role of endothelial cell receptor mediated transduction events in response to humoral inflammatory mediators; (ii) to examine the role of the endothelial cell in transduction of chemoattractant signals from interstitial sites to circulating leukocytes; and (iii) to examine the interaction of endothelial cells with neutrophils in the inflammatory response; and (iv) to examine the interaction of endothelial cells with pathogenic microorganisms. We found a rise in endothelial cytosolic calcium in response to the neutrophil-independent promoters of vascular permeability (histamine and platelet activating factor) whereas neutrophil-dependent promoters of increased vascular permeability (C5a, formyl-methionyl-leucyl-phenylalanine, and LTB_4) elicit no change in endothelial cytosolic calcium. We also documented histamine mediated changes in endothelial shape and monolayer integrity monitored by macromolecular diffusion across cultured endothelial monolayers. In other studies we demonstrated that endothelial cells bind formyl peptide chemoattractants, and release them intact. The characteristics of the binding site on endothelial cells differs in affinity and binding specificity from that found on phagocytic cells. The endothelial cell may direct transport of chemoattractant peptides from interstitial sites to the vascular lumen. In other studies we examined the interaction between fungal hyphae and endothelial cells, documenting a protective effect of neutrophils.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00495-01 LCI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Events in Mast Cell Secretion

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID
Others:	Robert J. Hohman, M.D.	Expert	LCI/NIAID
	Juan L. Rodriguez, M.D.	NRSA	LCI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.85	0.85	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Degranulation of mast cells and basophils is the fist step in a variety of allergic diseases. The rat basophilic leukemia (RBL) cell is valuable model system for studying the complicated sequence of events that begins with aggregation of the IgE receptor and ends with mediator release. The immediate goal of this project is to develop a method of permeabilizing cells in such a way as to maintain their ability to degranulate when challenged with antigen. The pore size must be small enough to maintain cell viability, yet big enough to permit small molecules (i.e., substrates and inhibitors of various enzymes) to have access to the cytoplasm. This will permit us to examine the effect on degranulation of compounds that normally are excluded by the plasma membrane, and will permit characterization of individual biochemical events leading to histamine secretion.

The method being developed employs alpha toxin, a protein with a molecular weight of 32 kD secreted from growing cultures of S. aureus. Upon binding to eukaryotic cells, the toxin forms a hexamer with five other toxin molecules and becomes inserted in the plasma membrane forming a pore of 2 to 3 nm spanning the plasma membrane.

Preliminary data demonstrate that: 1) alpha toxin is capable of permeabilizing RBL cells to molecules with a molecular weight of 500 or less daltons; 2) the permeabilized cells maintain their ability to release histamine in response to antigen; and 3) the antigen stimulated activation of permeabilized cells is less influenced by calcium in the incubation buffer than intact cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00496-01 LCI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Interactions between the Human Immunodeficiency Virus (HIV) and Herpesviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.M. Ostrove	Senior Staff Fellow, LCI, NIAID
OTHER:	K. Croen	Medical Staff Fellow, LCI, NIAID
	H. Gendelman	Expert, LMM, NIAID
	A. Rabson	Senior Staff Fellow, LMM, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues x ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The role of herpesviruses as possible cofactors in the progression and pathogenesis of the acquired immune deficiency syndrome (AIDS) is being investigated. AIDS patients possess a wide variety of herpes infections and we have been studying the interactions of these viruses in controlled laboratory experiments. Recombinant clones containing the long terminal repeat sequences (LTR) from the human immunodeficiency virus (HIV) were linked to the indicator gene chloramphenicol acetyltransferase (CAT) and CAT activity was used as a measure of stimulation of the HIV. Infection of cells harboring the HIV-LTR-CAT with herpes simplex virus types 1 and 2 (HSV) were able to stimulate CAT activity. The individual HSV genes responsible for this effect are being mapped. Analysis of deletion mutants within the HIV-LTR have helped map the regions of the LTR required for HSV activity. These experiments are being extended by the use of an infectious clone of HIV whose replication can be stimulated by HSV infection and various clones HSV genes. We are also examining clinical specimens by in situ hybridization for the presence of both HIV and herpes RNA and DNA sequences.

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LABORATORY OF IMMUNOGENETICS
1987 Annual Report
Table of Contents

Z01-AI Project Number		Page
Summary Report		7-1
00166-10	Characterization of Rabbit MHC Antigens -- Kindt	7-9
00168-10	Cell Surface Markers of Rabbit Lymphocytes -- Kindt	7-10
00169-10	Analysis of Murine and Human Transplantation Antigens and Genes -- Coligan	7-11
00170-10	Studies of Human Histocompatibility Antigens -- Long	7-12
00171-10	Genetic Studies of Rabbit Immunoglobulins and Other Serum Proteins -- Sogn	7-13
00173-10	Control of Gene Expression -- Max	7-14
00180-09	Properties of Transformed Rabbit Cell Lines -- Sogn	7-15
00352-05	Characterization of Cell Surface Molecules Important for Immune Function -- Coligan	7-16
00389-04	Molecular Genotype Analyses in HLA Recombinant Human Families -- Robinson	7-17

ANNUAL REPORT
LABORATORY OF IMMUNOGENETICS
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
October 1, 1986 to September 30, 1987

RESEARCH PROGRESS

The Laboratory of Immunogenetics investigates the multigene families that are involved in the control of immune function. Research emphasizes the structure and function of these genes and their products as well as mechanisms for regulation of these genes. The major histocompatibility complex, the T cell antigen receptor and the immunoglobulin gene complexes of several different species have been studied. Most recent investigations concern studies of disease models involving disfunction of the immune system, as well as studies of the role of MHC antigens and T cell receptor molecules in antigen presentation. A wide range of techniques at the molecular, serological and functional level are used in these investigations.

STUDIES OF THE MAJOR HISTOCOMPATIBILITY COMPLEX

Investigations of the major histocompatibility complex (MHC) have included studies of human histocompatibility antigens and their role in antigen presentation in virally infected cells. Studies of the rabbit MHC have included studies of expression of class I and class II genes and development of reagents and techniques to carry out histocompatibility typing in the rabbit and to develop antibody reagents to be used in studies of expression of the histocompatibility antigens in this species.

Human histocompatibility antigens. The immune response occurs only if a T lymphocyte has been able to recognize the foreign antigen in association with a self molecule encoded in the MHC. These self molecules expressed on antigen-presenting cells are the polymorphic class II MHC antigens. In man, three class II MHC antigens exist: HLA-DR, -DQ and -DP; each one consists of an alpha-beta chain dimer. The aim of this project is to define whether additional class II antigens exist and to analyze how class II antigens function in antigen presentation and in their interaction with T lymphocytes. Two new functional class II genes have been found: DZ alpha and DO beta. Their distinct pattern of expression suggests that they do not encode alpha-beta chain partners and that each one may correspond to a new class II antigen (Long, Rosen-Bronson). By DNA-mediated gene transfer into murine fibroblasts it was shown that an alpha chain from one subregion (e.g. DR) could assemble and be expressed at the cell surface together with beta chains from other subregions (DO, DP and DQ) and vice-versa (Sekaly). These mixed isotype pairs could contribute significantly to the nonallelic diversity of MHC class II antigens and to the repertoire of restricting elements that can be used in immune responses. Cytotoxic T cells (CTL) specific for measles virus-infected cells are predominantly class II-restricted. Transfection of the measles virus matrix and nucleocapsid genes into fibroblasts previously transfected with DR alpha and beta genes has led to two new findings: (1) class II-restricted CTL do not require intact cell surface viral antigens for recognition and lysis and, (2) newly synthesized cytoplasmic proteins can be presented by class II antigens. Thus, antigen-presentation pathways for class I and class II antigens may not be mutually exclusive (Sekaly, collaboration with S. Jacobson). Transfection experiments which introduced the human CD4 molecule into a murine hybridoma specific for a murine class I MHC antigen and DR antigen into the murine target cell provided strong evidence that a direct CD4-DR interaction occurs and results in enhanced T cell response.

Characterization of rabbit MHC antigens. The rabbit MHC locus, designated RLA, encodes class I and class II molecules similar to those encoded in the human and murine systems. The rabbit class I family contains only between 8 to 13 genes, so is smaller than its human or murine counterparts. In contrast with the other systems, a single class I protein has been found in association with the beta-2 microglobulin at the surface of cells from a rabbit T cell line RL-5. Structural data are now available for four cDNA clones, and the genes corresponding to three of these have been partially or completely sequenced. The gene 19-1 has been shown to encode the expressed class I proteins. Expression studies have been done in normal rabbit tissues using probes derived from these clones (Rebiere). One of the cDNA clones, designated pR27, is transcribed only in the T-cell line and in rabbit thymus, and at lower levels in lymphoid tissues containing T cells. Even though pR27 has, like the murine Tla gene product, an unusual splicing pattern, no structural homology to the Tla was seen and the specificity of their expression might be different. Therefore, pR27 most likely represents a novel T-cell specific class I gene. Two other genes, 19-1 and 5-2, were shown to be transcribed in all the tissues and cell lines studied. This differential pattern of expression observed among the rabbit class I genes will provide a system to study mechanisms involved in the regulation of expression of these genes. A search for sequences and factors involved in normal and tissue-specific expression are now in progress (Lubin). Studies of the class II genes revealed that there are rabbit genes corresponding to every gene observed in the HLA-D region (Kindt, LeGuern). Class II alpha genes corresponding to HLA-DR, -DP, -DQ, and -DZ, as well as the counterpart of the HLA-DOβ gene, have now been observed (Kulaga). A gene remnant has also been found in the rabbit genome, and consist of sequences corresponding to a class II α gene intron 4, followed by exon 4, flanked by direct repeats (Marche). Studies on the class II genes are now concentrating on the tissue-specific expression of these different genes.

Expression of class I MHC antigens in the hamster. Work continues on the nature and physiological role of class I major histocompatibility antigens in the hamster. A series of studies on virally-transformed hamster fibroblast cell lines has revealed a heterogeneous group of virally-directed or transformation-related changes in expression of class I MHC antigens but as yet no evidence exists that these changes are causally related to differences in tumorigenicity among these cell lines (Lewis, Sogn). A set of clones encoding class I antigens derived from a hamster genomic library have been evaluated and 18 unique clones have been identified. Which of these, if any, represents the major cell-surface transplantation antigens has been under study using cDNA cloning techniques, but the answer has yet to be determined.

Variation in class I antigen structure. Major histocompatibility complex encoded class I molecules are integrally involved in the presentation of viral antigens to cytotoxic T lymphocytes (CTL). The goal of these studies is to gain an understanding of the regulation of expression, and the functional and antigenic properties of these molecules. Research has focused on the fact that multiple class I molecules can be generated from single genes by alternative splicing of pre-mRNAs (Margulies, Handy). The alternative class I forms vary in expression in different cell types and in their ability to undergo phosphorylation. It is tempting to speculate that alternative splicing may influence function by controlling the phosphorylation of class I molecules. The complexity of the murine *H-2D* region was further underlined by the discovery of additional *D*-region encoded molecules in the H-2^q haplotype and the delineation of a new family of *D*-region molecules which suggests a strong role for unequal crossing over in the evolution of *D*-region genes (Lillehoj). Multiple species were examined for the expression of soluble class I molecules equivalent to the murine Q10 molecule. Only members of the order Perrissodactyla were found to have such a molecule suggesting that independent evolutionary events created class I genes of similar function (Lew). In the human, the cloning and sequence analysis of the HLA-A11 gene revealed that the close antigenic relationship of HLA-A3 and HLA-A11 was founded in their high structural homology and was likely related to a shared glutamine residue at position 62 (Biddison, Coligan). From this data, a predicted cross-reactive recognition by CTL between the rare HLA-A3 subtype, HLA-3.2, and HLA-A11 was confirmed and offered support for the critical role of amino acid positions 152 and 156 in CTL recognition of class I molecules.

MOLECULAR STUDIES OF THE IMMUNE RESPONSE IN THE LABORATORY RABBIT

The rabbit has proven in the past to be an excellent model for various diseases, including lung damage, herpes infection, diseases of the eye, syphilis, and most recently, it has been shown that the rabbit is a permissive host for HTLV-I infection. Recent investigations concern a disease model for crescentic glomerulonephritis in the rabbit and a study of HTLV-I and HIV infection in this species. It is proposed to use newly acquired knowledge and reagents gained in characterization of the gene families involved in immunologic function and reagents derived from these studies.

Viral infection of rabbit lymphoid cells. Rabbit cell lines have been obtained by viral transformation of lymphoid cells, placing primary tumors in culture, and by generation of fibroblast lines from skin punches. A number of these cell lines have been characterized for specific functional activities and for expression of genes relevant to the immune system (Kulaga). In an attempt to derive T-cell lines, rabbits were injected with human cells producing HTLV-I and the majority of these (4 of 5) seroconverted. Cell lines derived from rabbits treated in this fashion from other workers were characterized and it was possible to assign certain T-cell characteristics to these lines. Recent experiments have emphasized attempts to infect rabbit cell lines with the human AIDS virus. One cell line, RL-5, which has been extensively studied in this laboratory and that was derived by transformation with *Herpes ateles* and another cell line transformed with HTLV-I were shown to infect with HIV. This was shown by quantitative reverse transcriptase analysis, by Western blot analysis and by the ability of supernatants from the infected lines to reinfect susceptible human cell lines (Folks, Kulaga, Kindt). More complete characterization of infected cell lines will include Southern blot analyses to determine how the HIV genome has integrated into rabbit cell lines and Northern blot analyses to indicate which viral transcripts are being made, whether these are of normal size, and whether any transcript(s) are preferentially expressed. Because of the susceptibility of the HTLV-I transformed cell line to infection by HIV, attempts will be made to infect seroconverted HTLV-I rabbits with rabbit and human cell lines that are infected with HIV.

Surface markers of rabbit lymphocytes. Investigations of rabbit T lymphocytes have involved the use of monoclonal antibodies and cDNA probes to follow gene expression under normal and pathologic conditions. DNA probes for these studies were obtained from a cDNA library prepared using RNA from a rabbit cell line transformed with the virus Herpes ateles. Probes are now available for constant and variable region T cell receptor α and β chain genes and for class I T cell specific products. In addition, class II genes homologous to HLA-DR, -DQ, -DP, and -DZ have been cloned from rabbit libraries. In addition, probes for the human genes CD4 and CD8 have been obtained and are being tested for use in our studies of T cell subpopulations. These studies involve determination of the normal pattern of gene expression in resting rabbit lymphocytes and in lymphocytes stimulated with T cell mitogenic agents. In addition, a model for crescentic glomerulonephritis that is induced by formation of immune complexes is also under study in collaboration with investigators from the University of Michigan (Wiggins, Kulaga). It was shown that T cells infiltrate the glomerulus and after several days become positive for MHC class II genes and synthesize increased amounts of T cell receptor β chains. These studies have used antibodies directed against the class II antigens and markers for T cells and have been verified by Northern blot analyses of control and diseased kidneys.

GENETIC ANALYSES OF T CELL RECEPTOR GENES

In order to investigate the genetic control of human immune responses, the inheritance of several genes known to play important roles in a variety of immune processes has been analyzed in human families (Robinson). HLA and T cell receptor (TCR) genes have been examined by Southern blot analyses using specific DNA probes. A total of six restriction fragment length polymorphisms (RFLP) associated with the TCR α gene complex were identified including three for the C region and three for V gene segments. In addition to one polymorphism associated with the TCRβ C region gene segments, seven TCR β V RFLP detected with four different probes were examined. The results of these studies confirm the expected linkage of V and C gene segments for both TCR α and β gene complexes. Polymorphism detected in genomic DNA samples with any given probe and restriction enzyme combination was limited. In most cases, two polymorphic fragments that segregated as alleles were observed. No evidence for deletion or expansion of a V gene family was obtained. Differences in the number of fragments hybridizing with a given probe could be attributed to homozygosity or heterozygosity for observed polymorphisms. Examination of the combination of markers present in a haplotype revealed extensive heterogeneity at this level for both TCR α and β chain genes, 14 different combinations of the six TCR α markers and 12 combinations of the eight TCR β markers were observed in the 32 parental haplotypes examined. The observation that a series of markers each displaying few allelic forms are present in multiple combinations suggests that recombination has occurred frequently in both TCR α and β chain genes. Identification of individuals who inherited recombinant TCR genes supports this concept. These polymorphisms will provide useful markers that will facilitate linkage studies, mapping studies, genetic analyses of T cell function, and the means of evaluating the role of TCR genes in disease susceptibility.

CONTROL OF GENE EXPRESSION

The regulation of gene expression of B lymphocytes is being investigated using two genes as models: the human κ light chain immunoglobulin gene and the human J chain gene. The expression of the κ gene is regulated by a transcriptional enhancer located in the J-C intron. Evidence of *in vivo* interactions between the 5' region of the enhancer and B cell nuclear proteins was detected (Max, Gimble). More recently, *in vitro* interactions between the enhancer and nuclear proteins were examined (extracted from B cell nuclei) using an exonuclease protection method. These experiments define two binding domains in the enhancer: a 5' domain that includes an enhancer "core" element and an octamer consensus sequence (κE1); and a 3' domain that includes another occurrence of the octamer sequence (κE2). Using the exonuclease protection protocol as an assay, a protein that binds to the 3' domain is being purified. The partially purified protein has been used to characterize the binding site by *in vitro* DNAase I footprinting. To study features of gene regulation that may be common to genes specifically expressed in B cells, the human J chain gene is being investigated (Flanagan). A search for an enhancer in a 15 kb segment spanning the genomic J chain gene has not demonstrated a region able to support transcription from either an SV40 or a J chain promoter sequence. Sequence analysis of the J chain promoter revealed motifs resembling regions of immunoglobulin promoters and enhancers. To assess the functional significance of these motifs, the J chain promoter - with or without varying 5' deletions - was linked to a marker gene and an immunoglobulin enhancer and tested in transient transfection assays. These experiments suggest that, apart from these motifs, additional elements are important for the function of the J chain promoter *in vivo*. The investigation of this promoter will be continued by *in vitro* mutagenesis and further deletion experiments as well as by analysis of *in vitro* binding of B cell nuclear proteins. A collaboration was recently initiated with Drs. T Rado and J. Mestecky to study expression of the J chain *protein* using J chain cDNA constructs derived in our lab.

CHARACTERIZATION OF CELL SURFACE MOLECULES IMPORTANT FOR IMMUNE FUNCTION

Multiple molecules exist on the surface of lymphocytes that are important for development, activation and regulation of the immune response. Of prime importance in this regard is the clonotypic αβ heterodimer T cell receptor (TCR) that is noncovalently associated with the multicomponent T3 complex (Koning, Maloy). In order to study the expression of the TCR-T3 complex, rabbit anti-peptide antibodies specific for human TCR α and β chains and T3 δ-chains were prepared. With these reagents, it was possible to show that all of the α, β and CD3 chains present on the surface of lymphocytes are bound as a TCR-CD3 complex and that the two β chain isotypes (Cβ1 and Cβ2) were expressed in about equal amounts. In the mouse an anti-peptide antibody was used to define a completely new TCR-T3 complex. This was initially described on Lyt2$^-$,L3T4$^-$ thymocytes and were found to consist of a 35 Kd γ chain disulfide linked to a 45 Kd δ chain. This receptor is expressed prior to the αβ TCR in the developing thymus and appears to be representative of a distinct T cell lineage differentiating within the thymus. Additional studies detected this receptor on a novel population of Thy-1^+, $T3^+$, Ia^- dendritic epidermal cells (DEC). At least 60% of these cells were positive upon *in situ* staining with the anti-γ chain antibody. Cell lines derived from DEC were found to express three types of novel TCRs: "classical" γ-δ of 35 Kd and 45 Kd respectively, an apparent γ-γ homodimer and an as yet undescribed receptor that may contain Cγ4 protein. Hybridomas that express these receptors and that can be grown in large amounts are being used to analyze the structural and functional properties of these receptors. In addition to the TCR-T3 complexes, there are other cell surface molecules that appear to play a role in T lymphocyte signal transduction. One such molecule, TAP, a Ly-6 linked T-cell activating glycoprotein has been characterized and has been found to be similar, but distinct, from the Ly6.1E antigen.

LABORATORY OF IMMUNOGENETICS
Annual Report
October 1, 1986 to September 30, 1987

ADMINISTRATIVE REPORT

The period covered by this report was marked by several major changes in the composition of the Laboratory of Immunogenetics. The laboratory was begun in July 1977 with three senior scientists, Drs. Kindt, Sogn and Coligan. In this year two of these three tenured scientists have departed from the laboratory. Dr. Sogn has joined the National Cancer Institute in the Molecular Immunology Program and Dr. Coligan will leave the laboratory to assume a new position in the IRP as Chief of the Biological Resource Branch. This leaves Dr. Kindt as the sole tenured scientist. Drs. Long and Max are senior investigators and are currently being proposed for tenure. This year Dr. Jeffrey Gimble, Medical Staff Fellow, will leave the laboratory to assume a position at the Oklahoma Research Foundation with a joint appointment at the University of Oklahoma Medical School. Dr. Rafick Sekaly will leave to assume charge of an immunology unit at the University of Montreal. Dr. Margherita Bagnato left the laboratory to take a position in the Bureau of Biologics. These departures will be offset by the arrival of new laboratory members. David Karp from Washington University St. Louis and Delores Jaraquemada from the University of London will join the group of Eric Long. David Recker from the University of Michigan will join Ed Max and Mark Gordon from the Bowman Gray Medical School will join the group of Dr. Kindt. Mary Ann Robinson will assume the position of Expert in order to continue and expand her human genetic studies on the T-cell receptor genes and Dr. Ruth Lupu from the University of Tel-Aviv will join her in these studies.

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LABORATORY OF IMMUNOGENETICS
Annual Report
October 1, 1986 to September 30, 1987

HONORS AND AWARDS

Dr. Kindt has presented laboratory data in seminars at the University of Texas, Georgetown University, University of Virginia, Northwestern University and the Institut Pasteur in Paris and at the FASEB meeting in Washington and the T-Cell Receptor meeting in Keystone, Colorado. He has also presented data at the HLA-H2 Gene Cloning meeting that was held in Les Avantes, Switzerland and was selected as one of the organizing committee for the next meeting of this group. In addition, Dr. Kindt was co-chairman of a meeting held under NIH/CNRS auspices in Marseille concerning the topic of molecular immunogenetics. Dr. Kindt has served his final year as a member of the American Cancer Society Advisory Review Group and continues to serve on the study section for the Multiple Sclerosis Society. He was appointed Deputy Editor of the Journal of Immunology and continues to serve on the Editorial Board of the Journal of Experimental Medicine. In addition, he is a member of the Board of Scientific Counselors for Oncor, Inc. and Adjunct Professor at Georgetown University. Dr. Eric Long has presented data in seminars at the Institut Pasteur, at the University of California, San Francisco, and the University of North Carolina. In addition, he has presented laboratory data at the HLA-H2 Cloning Meeting, at the T-Cell Receptor meeting and at the 18th International Leukocyte Culture Conference in La Grande Motte, France. Dr. Long will serve a second term on the organizing committee of the HLA-H2 Gene Cloning Meeting. Dr. Mary Ann Robinson served as Workshop Chairman at the ASHI Meeting and will play a role in organizing the next meeting of this society. She was an invited participant at the T-Cell Receptor Meeting in Keystone, Colorado and presented laboratory data at the FASEB meeting. Dr. Edward Max continues to serve as Associate Editor for the Journal of Immunology. Dr. Coligan presented seminars at Indiana University, Harvard University, Yale University, the University of Pennsylvania and the Pasteur Institut and made presentations at the HLA-H2 Cloning Workshop in Les Avantes, Switzerland, the Fourth International Symposium on Peptides in Immunology in Las Vegas, the T-Cell Receptor meeting in Keystone, Colorado, the FASEB meeting in Washington, D. C., to FASEB Summer Conference in Immunology at Saxons River, Vermont, and the 18th International Leukocyte Culture Conference in La Grande Motte, France. Dr. Coligan was appointed Advisory Editor for Molecular Immunology and continues to serve as Immunochemistry Section Editor for Immunologic Research and Associate Editor of the Journal of Immunology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00166-10 LIG

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Rabbit MHC Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Thomas J. Kindt	Chief	LIG/NIAID
OTHER:	Marie Christine Rebiere	Visiting Fellow	LIG/NIAID
	Margherita Bagnato	Visiting Fellow	LIG/NIAID
	Henrietta Kulaga	Staff Fellow	LIG/NIAID
	Eric Long	Visiting Scientist	LIG/NIAID
	Ira Lubin	Staff Fellow	LIG/NIAID

COOPERATING UNITS *(if any)*

Patrice Marche, Christian LeGuern, and Annie LeGuern, Institut Pasteur, Paris; Katherine Knight, University of Illinois, Chicago.

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.6	2.4	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The rabbit MHC locus, designated RLA, encodes class I and class II molecules similar to those encoded in the human and murine systems. The rabbit class I family contains only between 8 to 13 genes, so is smaller than its human or murine counterparts. In contrast with the other systems, a single class I protein has been found in association with the beta-2 microglobulin at the surface of cells from a rabbit T cell line RL-5. Structural data are now available for four cDNA clones, and the genes corresponding to three of these have been partially or completely sequenced. The gene 19-1 has been shown to encode the expressed class I proteins. Expression studies have been done in normal rabbit tissues using probes derived from these clones. One of the cDNA clones, designated pR27, is transcribed only in the T cell line and in rabbit thymus, and at lower levels in lymphoid tissues containing T cells. Even though pR27 has, like the murine Tla gene product, an unusual splicing pattern, no structural homology to the Tla was seen and the specificity of their expression might be different. Therefore, pR27 most likely represents a novel T cell specific class I gene. Two other genes, 19-1 and 5-2, were shown to be transcribed in all the tissues and cell lines studied. This differential pattern of expression observed among the rabbit class I genes will provide a system to study mechanisms involved in regulation of expression of these genes. Search for sequences and factors involved in normal and tissue-specific expression are now in progress. Studies of the class II genes revealed that there are rabbit genes corresponding to every gene observed in the HLA-D region. Class II alpha genes corresponding to HLA-DR, -DP, -DQ, and -DZ, as well as the counterpart of the HLA-DOβ gene, have now been observed. A gene remnant has also been found in the rabbit genome, and consists of sequences corresponding to a class II α gene intron 4, followed by exon 4, flanked by direct repeats. Studies on the class II genes are now concentrating on the tissue-specific expression of these different genes.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00168-10 LIG

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Cell Surface Markers of Rabbit Lymphocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Thomas J. Kindt	Chief	LIG/NIAID
OTHER:	Henrietta Kulaga	Staff Fellow	LIG/NIAID
	Marie Christine Rebiere	Visiting Fellow	LIG/NIAID
	Mark Gordon	NRC Fellow	LIG/NIAID

COOPERATING UNITS *(if any)*
P.A. Cazenave, Institut Pasteur, Paris; Roger Wiggins, Department of Medicine, University of Michigan School of Medicine

LAB/BRANCH
Laboratory of Immunogenetics

SECTION
Immunogenetics Research Section

INSTITUTE AND LOCATION
NIAID, National Institutes of Health, Bethesda, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.2	1.0	1.2

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☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Investigations of rabbit T lymphocytes have involved the use of monoclonal antibodies and cDNA probes to follow gene expression under normal and pathologic conditions. DNA probes for these studies were obtained from a cDNA library prepared using RNA from a rabbit cell line transformed with the virus Herpes ateles. Probes are now available for constant and variable region T cell receptor α and β chain genes and for class I T cell specific products. In addition, class II genes homologous to HLA-DR, -DQ, -DP, and -DZ have been cloned from rabbit libraries. In addition, probes for the human genes CD4 and CD8 have been obtained and are being tested for use in our studies of T cell subpopulations. These studies involve determination of the normal pattern of gene expression in resting rabbit lymphocytes and in lymphocytes stimulated with T cell mitogenic agents. In addition, a model for crescentic glomerulonephritis that is induced by formation of immune complexes is also under study in collaboration with investigators from the University of Michigan. It was shown that T cells infiltrate the glomerulus and after several days become positive for MHC class II genes and synthesize increased amounts of T cell receptor β chains. These studies have used antibodies directed against the class II antigens and markers for T cells and have been verified by Northern blot analyses of control and diseased kidneys.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00169-10 LIG

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Analysis of Murine and Human Transplantation Antigens and Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	John E. Coligan	Senior Investigator	LIG/NIAID
OTHER:	Diane Handy	Staff Fellow	LIG/NIAID
	Gary Kikuchi	Guest Worker	LIG/NIAID

COOPERATING UNITS *(if any)*
W. Biddison, NINCDS, NIH; D. Margulies, LI, NIAID

LAB/BRANCH
Laboratory of Immunogenetics

SECTION
Membrane Antigen Structure Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.2	2.0	1.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Major histocompatibility complex encoded class I molecules are integrally involved in the presentation of viral antigens to cytotoxic T lymphocytes (CTL). The goal of these studies is to gain an understanding of the regulation of expression, and the functional and antigenic properties of these molecules. Research has focused on the fact that multiple class I molecules can be generated from single genes by alternative splicing of pre-mRNAs. The alternative class I forms vary in expression in different cell types and in their ability to undergo phosphorylation. It is tempting to speculate that alternative splicing may influence function by controlling the phosphorylation of class I molecules. The complexity of the murine H-2D region was further underlined by the discovery of additional D-region encoded molecules in the H-2^{q} haplotype and the delineation of a new family of D-region molecules which suggests a strong role for unequal crossing over in the evolution of D-region genes. Multiple species were examined for the expression of soluble class I molecules equivalent to the murine Q10 molecule. Only members of the order Perrissodactyla were found to have such a molecule suggesting that independent evolutionary events created class I genes of similar function. In the human, the cloning and sequence analysis of the HLA-A11 gene revealed that the close antigenic relationship of HLA-A3 and HLA-A11 was founded in their high structural homology and was likely related to a shared glutamine residue at position 62. From this data, a predicted cross-reactive recognition by CTL between the rare HLA-A3 subtype, HLA-3.2, and HLA-A11 was confirmed and offered support for the critical role of amino acid positions 152 and 156 in CTL recognition of class I molecules.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00170-10 LIG

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)
Studies of the Human Histocompatibility Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Eric O. Long	Visiting Scientist	LIG/NIAID
OTHER:	Rafick Sekaly	Visiting Fellow	LIG/NIAID
	Sandra Rosen-Bronson	Staff Fellow	LIG/NIAID
	Roberto Biassoni	Visiting Fellow	LIG/NIAID
	David Karp	Medical Staff Fellow	LIG/NIAID
	Christine Lee	Howard Hughes Medical Student	LIG/NIAID

COOPERATING UNITS (if any)
Steve Jacobson, Neuroimmunology Branch, NINCDS; Philippa Marrack, National Jewish Hospital, Denver, CO.

LAB/BRANCH
Laboratory of Immunogenetics

SECTION
Immunogenetics Research Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5.5	4.3	1.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The immune response occurs only if a T lymphocyte has been able to recognize the foreign antigen in association with a self molecule encoded in the major histocompatibility complex (MHC). These self molecules expressed on antigen-presenting cells are the polymorphic class II MHC antigens. In man, three class II MHC antigens exist: HLA-DR, -DQ and -DP; each one consists of an alpha-beta chain dimer. The aim of this project is to define whether additional class II antigens exist and to analyze how class II antigens function in antigen presentation and in their interaction with T lymphocytes. Two new functional class II genes have been found: DZ alpha and DO beta. Their distinct pattern of expression suggests that they do not encode alpha-beta chain partners and that each one may correspond to a new class II antigen. By DNA-mediated gene transfer into murine fibroblasts it was shown that an alpha chain from one subregion (e.g. DR) could assemble and be expressed at the cell surface together with beta chains from other subregions (DO, DP and DQ) and vice-versa. These mixed isotype pairs could contribute significantly to the nonallelic diversity of MHC class II antigens and to the repertoire of restricting elements that can be used in immune responses. Cytotoxic T cells (CTL) specific for measles virus-infected cells are predominantly class II-restricted. Transfection of the measles virus matrix and nucleocapsid genes into fibroblasts previously transfected with DR alpha and beta genes has led to two new findings: (1) class II-restricted CTL do not require intact cell surface viral antigens for recognition and lysis and, (2) newly synthesized cytoplasmic proteins can be presented by class II antigens. Thus, antigen-presentation pathways for class I and class II antigens may not be mutually exclusive (collaboration with S. Jacobson). Transfection experiments which introduced the human CD4 molecule into a murine hybridoma specific for a murine class I MHC antigen and DR antigen into the murine target cell provided strong evidence that a direct CD4-DR interaction occurs and results in enhanced T cell response.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00171-10 LIG

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic Studies on Rabbit Immunoglobulins and Other Serum Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John A Sogn	Senior Investigator	LIG/NIAID
OTHER:	Fathia Mami	Visiting Fellow	LIG/NIAID

COOPERATING UNITS *(if any)*
J. Coe, RML, A.M. Lewis, LIP, NIAID; P. Tucker, University of Texas Southwestern Medical School, Dallas; K. Roux, Florida State University; P.A. Cazenave, Institut Pasteur, Paris.

LAB/BRANCH
Laboratory of Immunogenetics

SECTION
Immunogenetics Research Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.7	1.3	1.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Work continues on the nature and physiological role of class I major histocompatibility antigens in the hamster. A series of studies on virally-transformed hamster fibroblast cell lines has revealed a heterogeneous group of virally-directed or transformation-related changes in expression of class I MHC antigens but as yet no evidence exists that these changes are causally related to differences in tumorigenicity among these cell lines. A set of clones encoding class I antigens derived from a hamster genomic library have been evaluated and 18 unique clones have been identified. Which of these, if any, represents the major cell-surface transplantation antigens has been understudy using cDNA cloning techniques, but the answer has yet to be determined. Another example of anti-allotype induced "latent allotypes" in the rabbit group a allotype system has been confirmed by sequence from another independent laboratory.

Studies of murine λ light chain genes in the wild derived mouse strain SPE have resulted in characterization of genes analogous to Cλ2, Cλ4, Cλ5 and Vλ2 in laboratory mice. The SPE Cλ2 gene apparently encodes a functional product whereas Cλ4 is obviously a pseudogene by virtue of large deletions in the coding sequence. Only a single Vλ gene with homology to Vλ1 or Vλ2 of laboratory mice was found for SPE. The analog of Cλ5 of C57 mice was studied in SPE and found to be highly conserved. In addition, Cλ5 sequences were seen in all wild mouse strains tested. The gene appeared to be present as a single copy even in strains that have greatly expanded Cλ1 or Cλ2 gene families.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00173-10 LIG

PERIOD COVERED

October 1, 1986 to September 20, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Control of Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Edward E. Max	Commissioned Officer	LIG/NIAID
OTHER:	Jeffrey Gimble	Medical Staff Fellow	LIG/NIAID
	James Flanagan	Medical Staff Fellow	LIG/NIAID
	David Recker	Medical Staff Fellow	LIG/NIAID

COOPERATING UNITS (if any)

Dr. T. Ley, Div. Hematology-Oncology, Jewish Hospital, St. Louis, MO; A. Rabson & H. Gendelman, Lab. Mol. Microbiol. NIAID, K. Ozato, Lab. of Develop. & Mol. Immunity, NICHHD; Drs. T. Rado and J. Mestecky, Univ. of Alabama at Birmingham.

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
4.5	3.3	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The regulation of gene expression of B lymphocytes is being investigated using two genes as models: the human κ light chain immunoglobulin gene and the human J chain gene. The expression of the κ gene is regulated by a transcriptional enhancer located in the J-C intron. We were able to detect evidence of in vivo interactions between the 5' region of the enhancer and B cell nuclear proteins. More recently, we have been examining in vitro interactions between the enhancer and nuclear proteins (extracted from B cell nuclei) using an exonuclease protection method. These experiments define two binding domains in the enhancer: a 5' domain that includes an enhancer "core" element and an octamer consensus sequence (κE1); and a 3' domain that includes another occurrence of the octamer sequence (κE2). Using the exonuclease protection protocol as an assay, we have been purifying a protein that binds to the 3' domain. The partially purified protein has been used to characterize the binding site by in vitro DNAase I footprinting. To study features of gene regulation that may be common to genes specifically expressed in B cells, we have been investigating the human J chain gene. A search for an enhancer in a 15 kb segment spanning the genomic J chain gene has not demonstrated a region able to support transcription from either an SV40 or a J chain promoter sequence. Sequence analysis of the J chain promoter revealed motifs resembling regions of immunoglobulin promoters and enhancers. To assess the functional significance of these motifs, the J chain promoter - with or without varying 5' deletions - was linked to a marker gene and an immunoglobulin enhancer and tested in transient transfection assays. These experiments suggest that, apart from these motifs, additional elements are important for the function of the J chain promoter in vivo. We are continuing the investigation of this promoter by in vitro mutagenesis and further deletion experiments as well as by analysis of in vitro binding of B cell nuclear proteins. We have recently initiated a collaboration with Drs. T Rado and J. Mestecky to study expression of the J chain protein using J chain cDNA constructs derived in our lab.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00180-09 LIG

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Properties of Transformed Rabbit Cell Lines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Thomas J. Kindt	Chief	LIG/NIAID
OTHER:	John Sogn	Research Chemist	LIG/NIAID
	Henrietta Kulaga	Staff Fellow	LIG/NIAID

COOPERATING UNITS *(if any)*
T.M. Folks, Laboratory of Immunoregulation, NIAID

LAB/BRANCH
Laboratory of Immunogenetics

SECTION
Immunogenetics Research Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.9	1.2	1.7

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rabbit cell lines have been obtained by viral transformation of lymphoid cells, placing primary tumors in culture, and by generation of fibroblast lines from skin punches. A number of these cell lines have been characterized for specific functional activities and for expression of genes relevant to the immune system. In an attempt to derive T-cell lines rabbits were injected with human cells producing HTLV-I and the majority of these (4 of 5) seroconverted. Cell lines derived from rabbits treated in this fashion from other workers were characterized and it was possible to assign certain T-cell characteristics to these lines. Recent experiments have emphasized attempts to infect rabbit cell lines with the human AIDS virus. One cell line, RL-5, which has been extensively studied in this laboratory and that was derived by transformation with Herpes ateles and another cell line transformed with HTLV-I were shown to infect with HIV. This was shown by quantitative reverse transcriptase analysis, by Western blot analysis and by the ability of supernatants from the infected lines to reinfect susceptible human cell lines. More complete characterization of infected cell lines will include Southern blot analyses to determine how the HIV genome has integrated into rabbit cell lines and Northern blot analyses to indicate which viral transcripts are being made, whether these are of normal size, and whether any transcript(s) are preferentially expressed. Because of the susceptibility of the HTLV-I transformed cell line to infection by HIV, attempts will be made to infect seroconverted HTLV-I rabbits with rabbit and human cell lines that are infected with HIV.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00352-05 LIG

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characterization of Cell Surface Molecules Important for Immune Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John E. Coligan	Senior Investigator	LIG/NIAID
OTHER:	Frits Koning	Visiting Fellow	LIG/NIAID
	Angel Ezquerra	Visiting Fellow	LIG/NIAID
	Tom McConnell	Guest Worker	LIG/NIAID
	Werner Lesslauer	Guest Worker	LIG/NIAID

COOPERATING UNITS *(if any)*
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LAB/BRANCH
Laboratory of Immunogenetics

SECTION
Membrane Antigen Structure Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.7	2.5	1.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Multiple molecules exist on the surface of lymphocytes that are important for development, activation and regulation of the immune response. Of prime importance in this regard is the clonotypic αβ heterodimer T cell receptor (TCR) that is noncovalently associated with the multicomponent T3 complex. In order to study the expression of the TCR-T3 complex, rabbit anti-peptide antibodies specific for human TCR α and β chains and T3 δ-chains were prepared. With these reagents, it was possible to show that all of the α, β and CD3 chains present on the surface of lymphocytes are bound as a TCR-CD3 complex and that the two β chain isotypes (Cβ1 and Cβ2) were expressed in about equal amounts. In the mouse an anti-peptide antibody was used to define a completely new TCR-T3 complex. This was initially described on Lyt2$^-$,L3T4$^-$ thymocytes and were found to consist of a 35 Kd γ chain disulfide linked to a 45 Kd δ chain. This receptor is expressed prior to the αβ TCR in the developing thymus and appears to be representative of a distinct T cell lineage differentiating within the thymus. Additional studies detected this receptor on a novel population of Thy-1$^+$, T3$^+$, Ia$^-$ dendritic epidermal cells (DEC). At least 60% of these cells were positive upon in situ staining with the anti-γ chain antibody. Cell lines derived from DEC were found to express three types of novel TCRs: "classical" γ-δ of 35 Kd and 45 Kd respectively, an apparent γ-γ homodimer and an as yet undescribed receptor that may contain Cγ4 protein. Hybridomas that express these receptors and that can be grown in large amounts are being used to analyze the structural and functional properties of these receptors. In addition to the TCR-T3 complexes, there are other cell surface molecules that appear to play a role in T lymphocyte signal transduction. One such molecule, TAP, a Ly-6 linked T-cell activating glycoprotein has been characterized and has been found to be similar, but distinct, from the Ly6.1E antigen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00389-04 LIG

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Genotype Analyses in HLA Recombinant Human Families

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mary Ann Robinson	Senior Staff Fellow	LIG/NIAID
	Thomas J. Kindt	Chief	LIG/NIAID

COOPERATING UNITS *(if any)*
D. Bernard Amos, Duke University; S. Hauser, Massachusetts General Hospital

LAB/BRANCH
Laboratory of Immunogenetics

SECTION
Immunogenetics Research Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.1	1.3	.8

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to investigate the genetic control of human immune responses, the inheritance of several genes known to play important roles in a variety of immune processes has been analyzed in human families. HLA and T cell receptor (TCR) genes have been examined by Southern blot analyses using specific DNA probes. A total of six restriction fragment length polymorphisms (RFLP) associated with the TCR α gene complex were identified including three for the C region and three for V gene segments. In addition to one polymorphism associated with the TCRβ C region gene segments, seven TCR β V RFLP detected with four different probes were examined. The results of these studies confirm the expected linkage of V and C gene segments for both TCR α and β gene complexes. Polymorphism detected in genomic DNA samples with any given probe and restriction enzyme combination was limited. In most cases, two polymorphic fragments that segregated as alleles were observed. No evidence for deletion or expansion of a V gene family was obtained. Differences in the number of fragments hybridizing with a given probe could be attributed to homozygosity or heterozygosity for observed polymorphisms. Examination of the combination of markers present in a haplotype revealed extensive heterogeneity at this level for both TCR α and β chain genes, 14 different combinations of the six TCR α markers and 12 combinations of the eight TCR β markers were observed in the 32 parental haplotypes examined. The observation that a series of markers each displaying few allelic forms are present in multiple combinations suggests that recombination has occurred frequently in both TCR α and β chain genes. Identification of individuals who inherited recombinant TCR genes supports this concept. These polymorphisms will provide useful markers that will facilitate linkage studies, mapping studies, genetic analyses of T cell function, and the means of evaluating the role of TCR genes in disease susceptibility.

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LABORATORY OF IMMUNOLOGY
1987 Annual Report
Table of Contents

Z01-AI Project Number		Page
Summary		8-1
00030-19	Antigen Recognition and Activation of Immunocompetent Cells - Paul	8-12
00035-12	Specificity in Immune Responses - Inman	8-13
00036-22	Ig Genetics: Ontogeny and Differentiation of Cells of the Rabbit Immune System - Mage	8-14
00147-11	The Mechanism of Activation of Thymus-Derived Lymphocytes - Schwartz	8-15
00148-11	Immunological Studies of Guinea Pig L_2C Leukemia - Green	8-16
00186-14	Pathogenesis of Autoimmunity in Inbred Strains in Mice - Chused	8-17
00203-08	Applications of Flow Cytometry - Chused	8-18
00223-06	Cellular Interactions in the Immune Response - Schwartz	8-19
00224-06	Monoclonal Antibodies as Probes for T Cell Activation - Shevach	8-20
00226-06	Rabbit Allotypes: Structure, Organization and Regulated Expression of Ig Genes - Mage	8-21
00349-05	Structure and Function of Murine Class II MHC Genes and Gene Products - Germain	8-22
00394-04	Molecular Genetic Analysis of Lymphocyte Function - Margulies	8-23
00403-04	Molecular Genetic Analysis of T Cell Receptor Structure and Repertoire - Germain	8-24

00425-03 Lymphocyte Physiology - Chused 8-25

00427-03 Antigen-Specific and Antigen-Nonspecific Cellular Cytotoxicity - Sitkovsky 8-26

00493-01 B Cell Stimulatory Factor-1 (BSF-1)/ Interleukin-4 (IL-4) - Paul 8-27

PHS-NIH
Summary Statement
Office of the Chief
Laboratory of Immunology
October 1, 1986 through September 30, 1987

Introduction

The Laboratory of Immunology is concerned with the elucidation of the fundamental mechanisms underlying immunologic responses. It had made rapid progress through the use of three new technologies which are creating a revolution in immunologic science. These are the use of monoclonal antibodies, the adaptation of techniques of molecular genetics to immunologic problems, and the use of long-term lines of cloned normal and transformed lymphocytes. The continued use and major improvement of these approaches should allow solution of many of the major problems which have concerned immunologists and should provide important advances in the efforts to more precisely regulate the normal and the disordered immune response.

Structural Model of Class II Major Histocompatibility Complex (MHC) Molecules

Class II MHC molecules are heterodimers of α and β chains that act as co-recognition elements in antigen recognition by T cells. Evidence has been obtained that class II molecules exert this function by binding to peptides derived from exogenous antigens, creating a complex recognized by the T cell receptor. Laboratory of Immunology scientists have developed a structural model for the functions of class II molecules based upon an analysis of mutant and natural polymorphic chains, using DNA-mediated gene transfer systems. Detailed study of the effects of varying the β chain of the I-A class II molecule (A_β) have led to the conclusions that residues in the third hypervariable region (HV3) of the N-terminal domain of the β chain are exposed on the surface of the class II molecule and that changes in these residues, while not impairing the overall structure, level of expression, or "pairing specificity" of the β chain, markedly alter its specificity as a co-recognition element. Thus, mutations in this region alter the capacity of the class II molecule to be co-recognized, with specific antigens, by T cells specific for the wild type molecule in association with the same antigen. By contrast, mutations in the amino terminal half of the first domain of the β chain of the I-A class II molecule ($A_{\beta 1}$) play a critical role in determining the specificity of pairing of the A_α and A_β chains or in regulating the transport of these chains to the membrane. It is proposed that the polymorphic residues of the amino-terminal of the A_α and A_β chains participate in an intimate interaction to allow formation of a normal class II molecule that can be efficiently transported to the membrane. In the intact class II antigen, these regions would be in the interior of the molecule and hence could affect immune recognition events only in an indirect manner by altering the conformation of the exposed regions. By contrast, the HV3 portion of the β chain appears to constitute the major site on the β chain that participates in the interaction of class II molecules with T cell receptors, antibodies, and, possibly, antigen-derived peptides. This model is in agreement with emerging data suggesting a single peptide-binding region and a single T cell restriction region in class II molecules (Braunstein, Ronchese and Germain, LI/NIAID).

Soluble Class I Major Histocompatibility Complex (MHC) Molecules Bind to Allospecific T Cell Hybridomas

T cell receptors recognize complexes consisting of peptides and class I or class II MHC molecules; some T cells appear to be specific for allogeneic class I or class II MHC molecules alone. The nature of these interactions has been difficult to study because both the T cell receptor and the antigens for which they are specific are membrane associated molecules. In an effort to develop a system through which the function and structure of T cell receptors and of class I MHC molecules could be more directly measured, a method has been derived for the expression of class I molecules as soluble proteins rather than as membrane associated molecules. The strategy used is based on the existence of the Q10 gene; this gene encodes a non-polymorphic class I molecule that is normally produced as a soluble protein. Its solubility is based on the structure of its carboxy terminal region. Through the creation of chimeric genes in which the exons coding for external domains are drawn from genes from conventional polymorphic class I MHC genes and in which exons for the carboxy-terminal portion of the molecule are drawn from Q10, a series of soluble class I proteins have been generated. These include $H\text{-}2D^d$, $H\text{-}2K^b$, $H\text{-}2L^d$, $H\text{-}2K^k$, $H\text{-}2K^{bm6}$, $H\text{-}2K^{bm10}$ and HLA-A2. Crystals are being prepared of some of these molecules for high resolution structure determination by X-ray crystallography. Some progress has been made in producing crystals but none of suitable size has yet grown. Purified monomeric $H\text{-}2D^d$/Q10 molecules bind to cells of a hybridoma line capable of responding to $H\text{-}2D^d$ cells. This directly demonstrates that such T cells bear receptors that recognize external domains of the D^d molecule. Monomeric D^d/Q10 fails to stimulate interleukin-2 (IL-2) production by these hybridomas but polymerized D^d/Q10 molecules can stimulate such production. These results indicate the direct specificity of receptors of allo-reactive T cells for that alloantigen itself and with allow conclusions as to energy of interactions. They form the basis of comparison though which the binding and stimulation by antigen-MHC complexes can be evaluted. Furthermore, the availability of large amounts of a variety of soluble class I molecules should allow structure determinations and should make possible careful structure function analyses for class I molecules in their interaction with T cells (Margulies, McCluskey, Lopez and Schneck, LI/NIAID).

Molecular Identification of Genetic Defects Regulating Levels of Expression of Kappa Light Chains

Basilea rabbits produce little or no immunoglobulin possessing light chains of the K1b9 type. In normal rabbits, this would be the predominant light chain type expressed. By contrast, Basilea rabbits produce elevated levels of K2 and of λ light chains. Laboratory of Immunology scientists have demonstrated that the low level of expression of the K1b9 chain is explained by a mutation in the 3' splice acceptor site in the J_κ-C_κ intron. Some splicing does occur because the mutation leads to the formation of an AAG 3' to the normal splice site but this splicing is inefficient and also generates a frameshift that results in premature termination of message translation (McCartney-Francis, Lamoyi and Mage). To extend this analysis, studies were initiated to determine why the K2 isotype of κ light chains is normally expressed at much lower levels than are κ chains of the K1 isotype. Sequences have been found in the J_κ-C_κ intron upstream of the enhancer of the K1 gene that are involved in the binding to the nuclear matrix. These sequences are deleted in the J_κ-C_κ intron of the K2 gene. This provides an opportunity to evaluate the significance of the nuclear matrix-binding sequences in the regulation of immunoglobulin gene expression (Mage, Hole, Max and Garrard).

T Cell Receptor α and β Chains Completely Determine T Cell Specificity for Antigen

T cell receptors contain α and β chains in association with the T3 antigen complex. These receptors determine the specificity of T cells, including the so-called "dual recognition" of antigen and major histocompatibility complex (MHC) molecules. It has been proposed that antigen and MHC recognition might be controlled by separate receptors. In order to examine this point, genes for the α and β chains of the T cell receptors of the mouse 2B4 hybridoma were isolated in an expressable form. This hybridoma recognizes a cytochrome C peptide in association with I-E^k class II molecules. To test whether, the 2B4 α and β chains were sufficient for the co-recognition of cytochrome C and I-E^k, the genes for these chains were transfected into the human Jurkat cell line or into Jurkat variants that failed to express endogenous α or β TCR genes. Transfection of the α and β genes alone led to the formation of mouse-human αβ heterodimers but failed to allow the transfected Jurkat cell to recognize cytochrome C presented by I-E^k-bearing cells, as judged by the production of interleukin-2 (IL-2). Only when the complete mouse αβ pair was expressed by the Jurkat cell was IL-2 produced in response to challenge with cytochrome C on I-E^k presenting cells. These results demonstrate that the antigen and MHC "co-specificity" of T cells is inherent in the αβ pair and that no additional receptors on that cell are required to explain histocompatibility restriction (Saito, Miller and Germain, LI/NIAID).

Dendritic Epidermal Cells (DEC) are T Cell Populations Enriched in the Expression of T Cell Receptors Composed of γ and δ Chains

The murine epidermis contains two major populations of hematopoietically derived cells; one, Langerhans cells, express class II major histocompatibility complex (MHC) molecules and lack Thy 1. The other cell type is negative for class II molecules but expresses Thy 1, a marker often associated with a T cell phenotype. Laboratory of Immunology scientists have prepared long term lines of these Thy 1^+ DEC. Two of these lines expressed abundant levels of mRNA for the T cell receptor (TCR) γ chain and fail to express mRNA for either the α or β chains of the TCR. Based on this finding, epidermal sheets were subjected to immunofluorescence staining with antibodies to the T3 ε chain and with an antiserum to the TCR γ chain. This revealed that all Thy 1^+ DEC expressed T3 and that 40-60% expressed surface γ chain, indicating that Thy 1^+ DEC constitute a T cell population markedly enriched in cells that utilize the γ chain in their TCR. A T cell hybridoma has been prepared from one of the Thy 1^+ DEC lines; this hybridoma expresses the TCR γ chain in association with another chain that is consistent with the TCR δ chain. The receptor complex consisting of γδ chains in a complex with T3 polypeptides displays the capacity to signal as shown by interleukin-2 production in such cells in response to anti-CD3 antibodies. These hybridomas provide a source of material for biochemical, genetic, and functional studies of the TCR δ chain. Furthermore, other Thy 1^+ DEC lines express γ chains in association with yet uncharacterized TCR chains. These results indicate that such cell lines will be of great value in the characterization of the range of TCR chains. Furthermore, the analysis of the Thy 1^+ DEC lines promises to be of great value in understanding the functional significance of T cells that bear receptors other than the conventional αβ heterodimer (Yokoyama and Shevach, LI/NIAID; Koning and Coligan, NIAID; Stingl, LI/NIAID and University of Vienna).

Elevation of Cytosolic Calcium ($[Ca^{2+}]_i$) in B Cell Activation

Cross-linkage of membrane immunoglobulin (Ig) on normal B cells leads to a rapid rise in $[Ca^{2+}]_i$ to concentrations as high as 1,500 to 2,000 nM from resting levels of 100 nM. This initial rise is followed by a fall to 300-400 nM, a concentration that is sustained for >6 hours, but only if anti-Ig continues to be present. The action of anti-Ig depends on the bivalency of the antibody and is partially inhibited by the cross-linkage of Fcγ receptors to membrane Ig, such as occurs when whole anti-Ig molecules are used. Activation of protein kinase C ablates the increase in $[Ca^{2+}]_i$ in response to anti-Ig as does elevation of cAMP. Antibodies to IgD appear to be more efficient than antibodies to IgM in stimulating elevation of $[Ca^{2+}]_i$, causing comparable increases at antibody concentrations that are 2-3 logs lower. By contrast, concentrations of anti-IgM and anti-IgD required for increased cell volume and increased expression of class II major histocompatibility complex molecules are quite similar. Laboratory of Immunology scientists have extended the analysis of the signalling pathway by preparing "signalling" variants of the B lymphoma line BAL 17. Mutagenized BAL 17 cells were selected for increased or diminished elevation of $[Ca^{2+}]_i$ in response to anti-IgM by flow cytometry and sorting. A "high" variant has been obtained that shows enhanced signalling in response to anti-IgM and, in addition, shows heightened levels of $[Ca^{2+}]_i$ and increased phosphatidyl inositol turnover in response to AlF_4. Since AlF_4 directly stimulates GTP-binding proteins in other cell types, the enhanced response to both signals suggests that GTP-binding proteins are involved in the response of B cells to receptor cross-linkage. In a "low" signalling mutant, surface IgM density is relatively normal as are responses to AlF_4, suggesting that this mutation affects a step distal to membrane Ig but proximal to GTP-binding proteins (Ishida, Ji and Chused, LI/NIAID; Finkelman, USUHS).

Polymerized Ligands Cause B Cell Signalling Through Membrane Immunoglobulin at Very Low Concentration

Antigens that bear repetitive epitopes are capable of stimulating antibody responses in T cell deficient animals, such as athymic mice, and, in vitro, in T cell depleted cultures. These antigens are referred to as type II antigens and constitute some of the biologically most important antigens, such as the capsular polysaccharides of pathogenic microorganisms. It is generally believed that the capacity of these molecules to elicit antibody responses in the presence of limited T cell influence is related to their heightened ability to cross-link membrane immunoglobulin (Ig) and thus to stimulate greater or qualitatively different biochemical signals within B cells. To study this point, Laboratory of Immunology scientists have prepared polymeric forms of anti-IgD antibodies using high molecular weight soluble carriers. The carriers used thus far have been high molecular weight dextrans and high molecular weight Ficoll. These polymers are highly effective in stimulating B cell responses as measured by ^{3}H-thymidine uptake. In some instances, polymerized anti-IgD gave responses equal to or in excess of monomer anti-IgD at 1,000-fold lower molar concentrations of monomer equivalent. These results strongly suggest that the nature and degree of cross-linkage on the cell surface are critical in determining the efficiency of signal generation in response to ligand-receptor binding. This system presents an ideal opportunity to test the immunon model of cell signalling (Inman, LI/NIAID; Mond, Finkelman, and Brunswick, USUHS; Dintzis, Johns Hopkins School of Medicine.

Phorbol Esters and Receptor Cross-Linkage Cause Desensitization of B Cell Receptors and Lead to the Phosphorylation of Discrete B Cell Proteins

Resting B cells display prompt increases in inositol phospholipid metabolism when their membrane receptors are cross-linked by anti-immunoglobulin antibody. This is accompanied by rapid increase in cytosolic calcium concentration ($[Ca^{2+}]_i$) and by protein phosphorylation mediated by protein kinase C (PKC). The importance of these events in the B cell activation process is emphasized by the finding that pharmacologic agents that elevate $[Ca^{2+}]_i$ (calcium ionophores) and that activate PKC (phorbol esters) acting jointly mimic many of the physiologic effects of anti-IgM on B cells. Laboratory of Immunology scientists have shown that pretreatment of B cells with phorbol esters inhibits the increase in $[Ca^{2+}]_i$ in response to anti-IgM. This appears to represent a receptor desensitization process since elevation of the earliest matabolite of phosphatidyl inositol bisphosphate is also blocked. The receptor desensitization phenomenon appears to involve a mechanism that is distal to membrane immunoglobulin itself since increased inositol phopholipid metabolism and elevation of $[Ca^{2+}]_i$ in response to AlF_4, a direct activator of nucleotide-binding protein, is also blocked. It seems most likely that the receptor desensitization phenomenon involves protein phosphorylation mediated by PKC. In parallel with these studies have been efforts to identify substrates of protein phosphorylation in B lymphocytes stimulated with anti-Ig antibodies or with phorbol esters. It has been shown that several membrane proteins and several proteins that are insoluble in non-ionic detergents are promptly phosphorylated in response to both stimulants. Of these, the most prominent has been identified as lamin B. Lamin B is a nuclear intermediate filament protein that becomes soluble when the nuclear membrane dissolves at the time of mitosis. It has been previously shown that lamin B is phosphorylated at the time of nuclear dissolution. These results indicate that lamin B is also phosphorylated in resting B cells in association with the activation of PKC and suggests that this phosphorylation may participate in the transmission of signals from the cell membrane to the nucleus in the regulation of new transcription (Mizuguchi, Hornbeck, Chused and Paul, LI/NIAID; Beaven, NHLBI; Huang, NICHD).

Thy-1 is a Signal Transducing Molecule in Lymphoid Cells

In addition to the T cell receptor (TCR) complex, several other surface molecules of lymphocytes have the capacity to transduce biochemical signals in T cells. These include CD2 and Tp44 in man and Thy 1 and components of the Ly6 complex in the mouse. Signalling through such molecules is dependent on cross-linkage by antibodies. Thy 1 is particularly interesting in this regard as it is a member of the immunoglobulin gene superfamily and virtually all monoclonal antibodies to Thy 1 stimulate a rise in cytosolic free calcium concentration ($[Ca^{2+}]_i$) in T cells and stimulate a proliferative response by T cells. In general, the elevation of $[Ca^{2+}]_i$ in response to anti-Thy 1 antibodies required that an antibody capable of further cross-linking the anti-Thy 1 be added and the proliferative response required a "second signal" that could be mediated by phorbol myristate acetate (PMA). Laboratory of Immunology scientists have demonstrated the critical role of Thy 1 as a signal transducing molecule by introducing the Thy 1 gene into the human T cell line Jurkat. Such cells also acquire the capacity to be stimulated to increased $[Ca^{2+}]_i$ by anti-Thy 1 antibodies and secrete interleukin-2 in response to anti-Thy 1 and PMA. To determine whether the signalling capacity of Thy 1 was dependent on the presence of the CD3/TCR complex, mutants of Jurkat cells that do not express this complex were transfected with Thy 1. Such Thy 1^+, $CD3/TCR^-$ cell lines showed increases in $[Ca^{2+}]_i$ in response to anti-Thy 1 although they failed to secrete interleukin-2. The reexpression of CD3/TCR on the surface of these cells, by transfection of a gene that complements the mutation in such cells, restored the capacity of anti-Thy 1 plus PMA to cause interleukin-2 production. These results indicate that Thy 1 can act as a signalling molecule independent of the CD3/TCR complex, but that full responsiveness of T cells to anti-Thy 1 does require the presence of the complex. A critical goal will be to establish the natural ligand for Thy 1 and to determine its physiologic role in regulation of T cell function (Shevach, Saito and Germain, LI/NIAID).

Exocytosis of Granules as a Novel Functional Assay of Cytotoxic T Cell Activation

Granule exocytosis was suggestted to occur, in association with the activation of cytotoxic T lymphocytes (CTLs), although the role of the contents of these granules in cytotoxicity has not been established. Laboratory of Immunology scientists have shown that a recently described trypsin-type esterase (BLT-esterase) preferentially expressed in CTLs is located in a low pH intracellular compartment and co-purifies with granules on Percoll gradients. This indicates that BLT-esterase is a component of CTL granules and that its enzymatic activity may be used as a biochemical marker of granule development and of granule exocytosis. Measurement of BLT-esterase activity thus provides a convenient assay of development and activation of CTL. Using this assay, Laboratory of Immunology scientists have shown that cross-linkage of T cell receptors for antigen (TCR) on CTL leads to granule exocytosis. The requirement for receptor cross-linkage can be bypassed by the use of protein kinase C activators, such as phorbol esters or bryostatins, together with calcium ionophores. The importance of elevation of cytosolic calcium in granule exocytosis is indicated by the finding that Ca^{2+} chelators block exocytosis as do Ca^{2+} channel blockers. Furthermore, specific calmodulin antagonists significantly inhibit TCR-triggered granule exocytosis, suggesting the importance of Ca^{2+}, calmodulin-dependent enzymes in the secretory process. Granule exocytosis can be inhibited by cholera toxin and by other agents that elevate intracellular cAMP such as forskolin and inhibitors of cAMP-phosphodiesterase. The direct involvement of the cAMP-dependent protein kinase in the inhibition of granule exocytosis is demonstrated by the combined inhibitory effect of the cyclic nucleotide analogs 8-Br-cAMP and N^6-benzoyl-cAMP; these agents are respectively specific for site I and site II of a regulatory subunit of the cAMP-dependent kinase. This assay system has also allowed the study of the relative roles of accessory molecules such as Lyt 2 in

CTL activation through TCR cross-linkage. Anti-Lyt 2 antibody blocks granule exocytosis in response to immobilized anti-TCR antibody, but has no effect on exocytosis mediated by a phorbol ester and a calcium ionophore. These results indicate that anti-Lyt 2 functions by blocking the interaction of the TCR with its target or by preventing signal generation at a proximal stage (Takayama, Trenn and Sitkovsky, LI/NIAID).

B Cell Stimulatory Factor-1/Interleukin-4 Regulates IgE Production In Vitro and In Vivo

B cell stimulatory factor-1 (BSF-1) has multiple effects on B cell function. It is now clear that BSF-1 promotes the secretion of IgG1 and IgE and evidence strongly suggests that it does so by regulating "switching" to the expression of these isotypes. Laboratory of Immunology scientists have recently shown that BSF-1 action in the class switching process can be mediated on the resting B cell itself. Incubation of resting B cells with BSF-1 alone for 2 days prepares cells to secrete IgG1 when stimulated with lipopolysaccharide (LPS) after removal of the BSF-1. This indicates that the effect of BSF-1 can be mediated independently of LPS and that it does not require cell division. It has further been shown that the effects of BSF-1 can be of very substantial magnitude. When high concentrations of BSF-1 are used, 50-70% of LPS stimulated cells express membrane IgG1 at 4 days of culture and ∿20% express membrane IgE. By means of flow cytometry and cell sorting, it has been shown that cells expressing membrane IgG1 are markedly enriched in precursors of IgG1 producers and depleted of precursors of IgM and IgG3 producers. B cells expressing membrane IgE are enriched in precursors of IgE production but are markedly depleted in precursors of IgM, IgG3, and IgG1 producers. A substantial fraction of IgE-bearing cells also express membrane IgG1; the Ig secretory potential of these cells and of IgE^-, $IgG1^+$ cells are now under intensive study as are the lineage relationship of the two cell types. Laboratory of Immunology scientists have shown that anti-BSF-1 antibody can block induction of IgE synthesis in vivo. Thus, mice that are infected with the larvae of the nematode Nippostrongylus brasiliensis normally show an increase in serum IgE levels from <0.1 µg/ml to >15 µg/ml. In the presence of monoclonal anti-BSF-1 antibody, this increased IgE production is reduced 10-fold. In another IgE-induction system, namely the injection of anti-IgD antibodies, the striking increases in IgE production that occur (∿100-fold) can be totally inhibited by administration of monoclonal anti-BSF-1 antibody. These results indicate that BSF-1 plays an important, perhaps critical, role in regulating IgE production. Together with recent results indicating that IgE and IgG1 production in response to LPS and BSF-1 are separately regulated, these results suggest that it may be possible to inhibit IgE production in vivo, without effecting IgG1 synthesis, thus forming the basis for the rational treatment of allergic disorders (Snapper, Ohara and Paul, LI/NIAID; Finkelman, USUHS).

Honors, Awards and Scientific Recognition

Laboratory of Immunology scientists play increasingly important roles in the U.S. and international immunological communities. Dr. William Paul served as president of the American Association of Immunologists during the past year and Dr. Ethan Shevach began a five year term as editor-in-chief of the *Journal of Immunology*. Dr. Ronald Germain was named to be principal organizer of the Sixth H-2/HLA cloning workshop and Dr. Shevach served as Vice-chairman of the Gordon Research Conference on Immunobiology and Immunochemistry. Dr. Ronald Schwartz was advanced to the position of chief of the new NIAID Laboratory of Cellular and Molecular Immunology.

Laboratory scientists serve on editorial boards of many scholarly publications. In addition to being editor-in-chief of the Journal of Immunology, Dr. Shevach is a member of the editorial boards of the *Proceedings of the Society for Experimental Biology and Medicine*, the *Journal of Immunological Methods* and *Cellular Immunology*. Dr. Paul is the editor of the *Annual Review of Immunology* and is an advisory editor of the *Journal of Experimental Medicine*, an associate editor of *Cell*, and a member of the editorial boards of *Immunological Reviews* and of the *Journal of Molecular and Cellular Immunology*. He is a member of the advisory editorial board of the *ISI Atlas of Science* and is consulting editor for immunology of the *Cecil Textbook of Medicine*. Dr. Germain is a deputy editor of the *Journal of Immunology*, and is a member of the advisory editorial board of the *Journal of Molecular and Cellular Immunology* and of the *Annales de L'Institut Pasteur*. Dr. Mage is a member of the editorial board of *Immunogenetics*. Dr. Inman is a member of the editorial board of *Analytical Biochemistry* and is an advisory editor of *Molecular Immunology*; he is a member of the editoral staff of the International Interest Group in Bioregulation Technology Science Newsletter. Dr. Chused is a member of the editorial board of *Cytometry*.

Dr. Paul is a member of the National Research Council's Board on Basic Biology and of its Committee on Research Opportunities in Biology. He is a member of the board of directors of the Federation of American Societies for Experimental Biology and is member of the selection committee for the Alfred P. Sloan, Jr. Prize of The General Motors Cancer Research Foundation. He is a member of the committee to visit the Division of Medical Sciences of the Board of Overseers of Harvard College, and of the advisory committee of the Pew Scholars Program in the Biomedical Sciences. Dr. Paul is a member of the scientific review board of the Howard Hughes Medical Institute, of the board of directors of the Foundation for Advanced Education in the Sciences, of the board of scientific advisors of the Jane Coffin Childs Memorial Fund for Medical Research, of the board of scientific consultants of the Memorial-Sloan Kettering Cancer Center, and of the scientific advisory committee of the Cancer Research Institute. He is chairman of the advisory committee of the Harold C. Simmons Arthritis Research Center.

During the past year, Dr. Paul gave the Rosalind Russell Memorial Lecture at the University of California, San Francisco, the Carl Moore Lecture at the Washington University School of Medicine, the Richard Gershon Lecture at Yale University, and the Erwin Neter Lecture at the annual meeting of the American Society for Microbiology. He presented the Presidential Address at the annual meeting of the American Association of Immunologists. Dr. Paul gave invited lectures at the annual meeting of the New England Immunology Group, at the meeting of the American Society for Cell Biology and at the annual meeting of the Brazilian Society for Immunology. He was an invited speaker at the symposium honoring the centenary of the founding of the Pasteur Institute and of the National Institutes of Health and at both the 18th Leucocyte Culture Conference and the Gordon Conference on Immunobiology and Immunochemistry.

Dr. Shevach was chairman of a minisymposium at the annual meeting of the American Association of Immunologists, and was an invited lecturer at the annual meetings of the Brazilian Society for Immunology, and of the American Association of Neuropathologists. He chaired a workshop at the UCLA Conference on the T Cell Receptor, and was an invited lecturer in course on the Biology of Parasitism at the Woods Hole Marine Biology Laboratory.

Dr. Germain is a member of the Advisory Committee on Clinical Investigations I (Immunology and Immunotherapy) of the American Cancer Society and is a member of the NIAID Clinical Review Subpanel. He was chairman of a session and was an invited speaker at the Gordon Conference on Immunochemistry and Immunobiology. He presented a lecture at an NIAID-sponsored plenary session at the annual meeting of the Federation of American Societies of Experimental Biology and was an invited lecturer at the meeting on Antigen Presenting Cells: Diversity, Differentiation and Regulation.

Dr. Mage was elected to fellowship in the American Association for the Advancement of Science and serves as the American Association of Immunologist's representative to the American Type Culture Collection.

Dr. Margulies was advanced to tenure status in the Laboratory of Immunology. He is a member of the Immunology and Immunotherapy Advisory Committee of the American Cancer Society. He presented an invited lecture at the Fifth H-2/HLA Cloning Workshop at Les Avants Switzerland, and presented a Director's seminar at the National Institutes of Health.

Dr. Sitkovsky gave invited presentations at the New York Academy of Sciences International Conference on Cytotoxic T Cells and the UCLA Symposium on the T Cell Receptor.

Administrative, Organizational and Other Changes

During the past year, Dr. Ronald Schwartz was appointed chief of the newly created NIAID Laboratory of Cellular and Molecular Immunology and Dr. Ira Green accepted a position as Head, Preclinical Trials in the Biological Response Modifiers Program of the National Cancer Institute. These two eminent scientists had been senior scientists in the Laboratory of Immunology for 11 and 19 years, respectively. Their contributions to the development of the Laboratory of Immunology have been enormous. Dr. David Margulies was advanced to tenure status in the Laboratory of Immunology. He had been appointed to a tenure track position in 1983; this promotion represents the recognition by peers at NIH of his excellent work.

The Laboratory continues to be a major training center for young scientists. During the past year Drs. Mark Avigan, Ned Braunstein, Melissa Brown, Christine Hua, Edmundo Lamoyi, James McCluskey, Kathleen Melez, and Junichiro Mizuguchi completed post-doctoral training periods in the Laboratory of Immunology and Mr. Richard Lopez completed a two year appointment as a Howard Hughes Medical Institute-NIH Scholar. Each of these scientists made an important contribution to the Laboratory research program and to the progress of Immunology.

During the past year, several post-doctoral fellows joined the Laboratory for research training. They included Drs. Ulus Atasoy, Geoffrey Chazen, Anne Fortier, Rosemarie Hunziker, Steven Kozlowski, Corine Layet, Graham LeGros, Gillis Otten, Kevan Roberts, Jane Ryan and Rolf Taffs. In addition, Drs. S. Z. Ben-Sasson (The Hebrew University of Jerusalem), Zvi Grossman (Tel-Aviv University), and Marshall Plaut (Johns Hopkins School of Medicine) came to the Laboratory on sabbatical visits.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00030-19 LI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Antigen Recognition and Activation of Immunocompetent Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	William E. Paul	Chief	LI, NIAID
Others:	J. Mizuguchi	Visiting Associate	LI, NIAID
	W. Tsang	Medical Staff Fellow	LI, NIAID
	P. Hornbeck	Staff Fellow	LI, NIAID
	D. Margulies	Senior Investigator	LI, NIAID
	T. Chused	Senior Investigator	LI, NIAID

COOPERATING UNITS *(if any)*

LC, NHLBI (M. Beaven).

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.1	2.1	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Cross-linkage of membrane immunoglobulin (Ig) on resting B lymphocytes leads to cellular activation. Biochemical correlates of this cellular activation that have been identified are rapid accumulation of inositol-1,4,5 trisphosphate, rapid increase in cytosolic free calcium concentration $[Ca^{2+}]_i$ and striking enhancement in protein kinase C (PKC)-mediated protein phosphorylation. These early biochemical events appear to be important in B cell activation because mimicking them with pharmacologic agents causes B cell activation. We have recently demonstrated that phorbol myristate acetate treatment of B cells blocks signalling normally generated by receptor cross-linkage. Such cells fail to show increased inositol phospholipid metabolism or to elevate $[Ca^{2+}]_i$, indicating that their receptors have been desensitized. These cells are not activated as a result of receptor-cross linkage, providing further support for the importance of these biochemical events in cellular activation.

Studies of the substrates of PKC revealed that one of the principal proteins phosphorylated by PMA-treatment of resting B cells was the nuclear intermediate filament protein lamin B. The rapid phosphorylation of a nuclear protein by PMA and by receptor cross-linkage suggests a mechanism through which receptor mediated signals may be communicated to the nucleus.

The structural basis of signalling is being studied through the creation of chimeric genes involving exons of class I major histocompatibility complex genes and of Ig genes. These genes have been transferred into B lymphomas and have been shown to be expressed under the influence of interferon. Studies of the capacity of these chimeric membrane proteins to signal upon cross-linkage are now in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00035-12 LI

PERIOD COVERED

October 1, 1986 to September 31, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Specificity in Immune Responses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. K. Inman Senior Investigator LI, NIAID

COOPERATING UNITS *(if any)* Dept of Medicine, USUHS (J. Mond, F. Finkelman, M. Brunswick); Dept of Biophysics, Johns Hopkins University School of Medicine, Baltimore, MD (H. M. Dintzis).

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.8	1.0	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

One important aim of this project is to explore the property of general multispecificity of antibodies and other kinds of receptors. Studies are in progress to compare the frequency distribution of binding constants of monoclonal antibodies for large numbers of diversely structured substances. Additional studies will be directed at showing to what extent binding events from the above trials are representations of alternative modes of complementation in the binding site region of a given receptor. These findings will be related to specificity and other important functions of receptor-effector interactions and networks in the immune system.

Another major effort of this project is directed toward the design and synthesis of highly specific and powerful immunomodulators. The current design principle is based on the signal amplification produced by polyvalent presentation of effector molecules and is closely allied with the immunon construct of Dintzis et al. Effector molecules (e.g., monoclonal antibodies directed against specific cell surface proteins) are multiply and covalently linked to soluble polymer carriers of very high molecular weight using heterobifunctional reagents. Various structural parameters are being varied in order to optimize the performance of these immunomodulators, and collaborative studies are in progress to explore their properties. So far these studies have dealt mainly with mitogenic stimulation of B cells; preliminary experiments also have shown effective stimulation of T cells with a polymeric signal.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00036-22 LI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Ig Genetics: Ontogeny and Differentiation of Cells of the Rabbit Immune System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. G. Mage	Senior Investigator	LI, NIAID
Others:	B. Newman	Guest Researcher	LI, NIAID
	N. Hole	Visiting Fellow	LI, NIAID
	N. Harindranath	Visiting Fellow	LI, NIAID
	N. McCartney-Francis	Senior Investigator	LMI, NIDR
	E. Max	Senior Investigator	LIG, NIAID

COOPERATING UNITS (if any) Andrew S. Kelus, Basel Institute for Immunology, Basel, Switzerland; W. T. Garrard, Dept Biochemistry, Univ. Texas, Dallas, TX; Edmundo Lamoyi, National Univ. Mexico, Mexico City; J.-C. Jaton, Dept Biochemistry, Univ. Geneva, Geneva, Switzerland.

LAB/BRANCH
Laboratory of Immunology

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INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN YEARS	PROFESSIONAL	OTHER
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We use classical immunogenetic techniques as well as techniques of molecular biology to study the genetics of rabbit immunoglobulins (Igs) and T cell receptors and the regulated expression of the genes that encode these molecules. Basilea rabbits carry a mutation that results in loss of expression of the normal major Ig light chain type (K1b9). We showed that the 3' acceptor splice site of the Jκ-Cκ intron of the κ1b9 gene in Basilea rabbits has a G to A transition that results in the use of an AAG/ generated by the mutation and located one nucleotide downstream. The splicing generates a frameshift that results in premature termination of message translation. In addition, the splicing is probably inefficient in that much of the detectable K1 mRNA is not completely processed. These data provide strong support for our earlier suggestion that the splice site mutation accounts for the mutant Basilea phenotype (non-expression of K1b9 light chains). We are investigating why rabbits produce only trace amounts of Igs with light chains of the K2 isotype. We can detect mRNA encoding such light chains in splenocytes from rabbits infected with Trypanosoma equiperdum albeit at 1/100 to 1/1000 the levels in comparable mRNA preparations from Basilea rabbits. We found DNA fragments that bind to the nuclear matrix in a region of the Jκ-Cκ intron upstream of the enhancer that is deleted in the gene for K2 light chains. The functional significance of this deletion can now be evaluated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00147-11 LI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Mechanism of Activation of Thymus-Derived Lymphocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. H. Schwartz	Senior Investigator	LI, NIAID
OThers:	M. Jenkins	Guest Worker	LI, NIAID
	H. Quill	Staff Fellow	LI, NIAID
	K. Ogasawara	Visiting Fellow	LI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID,NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00148-11 LI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunological Studies of Guinea Pig L_2C Leukemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	I. Green	Senior Investigator	LI, NIAID
Others:	E. Gregg	Visiting Fellow	LI, NIAID

COOPERATING UNITS *(if any)* LCB, NCI (V. Hearing); LIG, NIAID (L. Maloy); Medicine Branch, NCI (D. Longo & S. Bridges); Tenovus Research Laboratory, Southampton General Hospital, Southampton, England (F. Stevenson); Richard Youle, NINCDS, SNB.

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Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00186-14 LI

PERIOD COVERED

October 1, 1986 to September 31, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis of Autoimmunity in Inbred Strains in Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. M. Chused	Senior Investigator	LI, NIAID
Others:	E. M. Brown	Biologist	LI, NIAID

COOPERATING UNITS *(if any)*

Renu Lal, Visiting Fellow, LPD, NIAID; John Coligan, Chief, BRB, NIAID; Philip J. Baker, Section Chief, LMI, NIAID; Herb Cooper, Section Chief, NCI.

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INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.1	.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The genetic control and immunologic mechanisms of autoimmune disease is being investigated in the New Zealand strains of mice, their F_1 hybrids, and recombinant-inbred lines derived from them.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00203-08 LI

PERIOD COVERED
October 1, 1986 to September 31, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Applications of Flow Cytometry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. M. Chused	Senior Investigator	LI, NIAID

COOPERATING UNITS *(if any)*

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Laboratory of Immunology

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INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.1	.1	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Flow cytometry is being used to analyze the differentiation of thymic lymphocytes. Instrumentation is being enhanced to permit simultaneous five and six parameter immunofluorescence.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00223-06 LI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cellular Interactions in the Immune Response

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. H. Schwartz	Senior Investigator	LI, NIAID
Others:	D. Pardoll	Medical Staff Fellow	LI, NIAID
	C. Hua	Visiting Fellow	LI, NIAID
	B. Fox	Guest Worker/Staff Fellow	LI, NIAID
	K. McCoy	Guest Worker/Staff Fellow	LI, NIAID

COOPERATING UNITS *(if any)* LMI, NIAID, B. J. Fowlkes, R. Asofsky, and BRMP; NCI, A. Kruisbeek; LI, R. N. Germain

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NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

PHS 6040 (Rev. 1/84) GPO 904-917

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00224-06 LI

PERIOD COVERED

October 1, 1986 to September 31, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Monoclonal Antibodies as Probes for T Cell Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. M. Shevach	Senior Investigator	LI, NIAID
Others:	W. Yokoyama	Medical Staff Fellow	LI, NIAID
	G. Pereira	Visiting Associate	LI, NIAID
	K. Roberts	Visiting Fellow	LI, NIAID
	K. Ono	Visiting Fellow	LI, NIAID
	D. Presky	Guest Worker	LI, NIAID
	K. Melez	Guest Worker	LI, NIAID

COOPERATING UNITS *(if any)* Dept of Dermatology, University of Vienna, Vienna, Austria (G. Stingl); Lab of Immunogenetics, NIAID, NIH (J. Coligan); Lab of Theoretical Biology, NCI, NIH (C. D. V. Black)

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NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN YEARS	PROFESSIONAL	OTHER
7.0	5.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major objective of our studies is to characterize cell surface structures on T lymphocytes which, in addition to the antigen specific receptor (Ti), are involved in the process of lymphocyte activation. We have defined the Thy-1 antigen as one of the critical signal transducing molecules in murine lymphoid cells. Transfection studies of the murine Thy-1.2 gene into the human T cell Jurkat have demonstrated that Thy-1 mediated T cell activation requires co-expression of the CD3 (T3)/Ti complex. We have combined studies using monoclonal antibodies (mAbs) with biochemical and molecular studies of T cell receptor (TCR) gene expression and demonstrated that Thy-1$^+$ dendritic epidermal cells (Thy-1$^+$ DEC), a unique population of cells within the murine epidermis, express the CD3 complex in associated with the products of the TCR γ- and δ-chain genes. Lastly, we have developed a rapid, quantitative model for the assessment of a number of parameters of lymphocyte activation in vivo. We have used this assay system to examine the effects of the immunosuppressive drug, Cyclosporin A (Cy A), on the development of lymphocyte activation in vivo and demonstrated that mechanism of action of Cy A in vivo may be distinct from its mechanism of action in vitro.

PHS 6040 (Rev. 1/84) GPO 904-917

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00226-06 LI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Rabbit Allotypes: Structure, Organization and Regulated Expression of Ig Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. G. Mage	Senior Investigator	LI, NIAID
Others:	B. Newman	Guest Researcher	LI, NIAID
	N. Hole	Visiting Fellow	LI, NIAID
	N. Harindranath	Visiting Fellow	LI, NIAID

COOPERATING UNITS *(if any)* Basel Institute for Immunology, Basel, Switzerland (Andrew S. Kelus); National University of Mexico, Mexico City (Edmundo Lamoyi); National Institute of Animal Industry, Ibaraki, Japan (Masanori Komatsu).

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NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We study genes of the rabbit immune system by techniques of molecular biology. We used a restriction fragment length polymorphism (RFLP) of the rabbit T cell receptor β chain constant region genes (Cβ) and an RFLP of rabbit immunoglobulin (Ig) kappa light chains (Cκ) to determine that rabbit Cβ and Cκ are not genetically linked as they are in mice, but segregate independently as in man. The Cκ RFLP on the other hand is closely linked to the expression of the K2basl Ig allotype and to a newly discovered RFLP of rabbit Vκ genes. No recombinants have been identified to date. We found evolutionary conservation of both sequence and linkage relationships of gene segments encoding T cell receptor β chain variable regions. Two of three closely linked rabbit Vβ genes have homologs that are closely linked in both mouse and man. A third rabbit Vβ gene hybridizes strongly to human DNA. The rabbit probe should identify and allow mapping of a previously unidentified human Vβ gene. Like other species, some rabbits have two similar Cβ genes but we found that others have three and allotypic forms of both Cβ1 and Cβ2. Stretches of alternating purines and pyrimidines 5' of Cβ1 exon 1, a perfect Chi sequence in exon 1, and Chi with one mismatch in the intron 3' of Cβ1 exon 1, surrounding the allotypic positions, may have contributed to the generation of similar allotypic amino acid sequences in both Cβ1 and Cβ2 of individual rabbits (isoallotypes) by a gene-conversion-like mechanism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00349-05 LI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Structure and Function of Murine Class II MHC Genes and Gene Products

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. Germain	Senior Investigator	LI, NIAID
Others:	N. Braunstein	Medical Staff Fellow	LI, NIAID
	A. Sant	Guest Worker	LI, NIAID
	J. Miller	Guest Worker	LI, NIAID
	F. Ronchese	Visiting Fellow	LI, NIAID
	J. Tou	Research Technician	LI, NIAID

COOPERATING UNITS (if any) LIG, NIAID (E. Long); LVD, NIAID (B. Moss); MC, NCI (J. Berzofsky); LCMI, NIAID (R. Schwartz); Washington Univ. School of Medicine (S. Cullen).

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NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 5.0	PROFESSIONAL: 4.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Class II MHC (Ia) gene products play critical roles in a variety of T lymphocyte responses. A combination of immunological, molecular genetic, and biochemical approaches is being used to study the relationship between Ia structure and function.

Ia expression has been examined following transfer of class II genes into a variety of recipient cells. These experiments have shown that polymorphic regions of the Ia molecule play a critical role in regulating membrane expression. For the β chain, irrespective of the α chain origin, allelically variable residues in the amino-terminal 50 amino acids control the level of surface expression. This control primarily affects the efficiency of transport, not the initial pairing of the chains. Invariant chain (Ii) is not required for this transport event.

Dissection of Ia structure-function relationships has revealed a segmental structure for these molecules. Distinct variable subregions participate differently in either control of expression, binding and display of antigenic peptides, or interaction with the T cell receptor. In particular, a single amino-acid change at position 29 in the Eβ chain alters the presentation of pigeon cytochrome c without affecting general properties of T cell recognition of the I-E molecule. Changes in positions 67, 70, or 71 of the Aβ chain, however, affect almost all T cell responses to the I-A molecule.

The cell biology of antigen processing and presentation by MHC molecules has been studied and a special role for the endosome/trans-Golgi low pH compartments demonstrated. The rules controlling class I vs class II MHC antigen presentation have been examined, with the data indicating that the way antigen enters a cell may determine the class of MHC molecule used for presentation.

These studies are providing new insight into the rules governing T cell co-recognition of antigen and MHC molecules, and the biochemistry and cell biology of protein multimer synthesis and transport.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00394-04 LI

PERIOD COVERED

October 1, 1986 to September 31, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetic Analyis of Lymphocyte Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David H. Margulies	Senior Investigator	LI, NIAID
Others:	J. McCluskey	Visiting Scientist	LI, NIAID
	L. Boyd	Chemist	LI, NIAID
	R. Lopez	Guest Worker	LI, NIAID
	P. Cohen	Guest Worker	LI, NIAID
	J. Schneck	Medical Staff Fellow	LI, NIAID
	R. Hunziker	Biologist	LI, NIAID

COOPERATING UNITS *(if any)*

LI, NIAID (J. Inman); LIG, NIAID (D. Handy and J. Coligan); IB, NCI (Terry Munitz and A. Singer); LMB, NIADDK (D. Davies and E. Padlan).

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TOTAL MAN-YEARS: 6.5	PROFESSIONAL: 2.7	OTHER: 3.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The ongoing focus of the laboratory is to understand in detail the structure/function relationships of cell surface molecules involved in the immune response. In particular, we have made significant progress over the past year in several areas: A. exploting molecular genetic techniques to generate a panel of cell lines expressing soluble counterparts of the murine class I major histocompatibility antigens H-2K^b, H-2D^d, H-2L^d, and H-2K^k; as well as the H-2K^b mutant H-2K^{bm10}; B. using the purified soluble molecules from H-2D^d and H-2K^b to attempt to grow crystals for X-ray crystallography; C. using the purified soluble molecules to study the biochemical and functional behavior of the H-2D^d molecule; D. analysis of the expression and function of in vitro lariat branch point mutations expected to control the choice of splice acceptor sites for the eighth exon of H-2K^b and H-2D^d; E. testing hypotheses about tolerance and T cell education by generating transgenic mice that express the soluble counterpart of the H-2D^d and H-2K^b genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00403-04 LI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetic Analysis of T Cell Receptor Structure and Repertoire

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. N. Germain	Senior Investigator	LI, NIAID
Others:	T. Saito	Visiting Fellow	LI, NIAID
	M. Avigan	Medical Staff Fellow	LI, NIAID
	R. Schwartz	Senior Investigator	LCMI, NIAID
	D. Pardoll	Medical Staff Fellow	LCMI, NIAID
	E. Shevach	Senior Investigator	LI, NIAID
	G. Stingl	Guest Worker	LI, NIAID

COOPERATING UNITS *(if any)*

LMI, NIAID (B. J. Fowlkes); BRMP, NCI (A. Kruisbeek, J. Ashwell); LIG, NIAID (J. Coligan); IB, NCI (J. Bluestone).

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Laboratory of Immunology

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INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN YEARS	PROFESSIONAL	OTHER
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

T lymphocytes divide and differentiate when they receive an appropriate activation signal. This signal can be provided by a clonally-distributed, specific antigen receptor (Ti-T3), or through certain broadly distributed cell membrane structures. Molecular biological and in vitro cell culture techniques have been used to investigate the expression of the genes encoding these activation structures, and to study the relationship between structure and function.

We have shown using DNA-mediated gene transfer that the clone-specific αβ heterodimer completely defines the dual antigen and MHC molecule specificity of mature peripheral T cells. Additional transfection studies have revealed what appears to be a relatively independent contribution of the two V regions of this single receptor to the dual specificity, raising the possibility that the germline repertoire of receptor gene segments is preselected for certain contributions to the ultimate receptor specificity, and that the two chains contribute in a different way to the receptor's recognition potential.

Studies of T cell receptor gene expression during ontogeny or T cell maturation in the mature thymus suggest that repertoire selection occurs intrathymically. These studies also have led to the identification of a new subset of T cell with a distinct receptor termed γδ. Analysis of long term lines of these latter cells shows them to have many of the same effector functions as αβ-expressing T cells, but their specificity remains unknown.

Investigation of the mechanism of activation of T cells by monoclonal antibodies to non-Ti-T3 surface structures such as Thy-1 revealed a strict dependence on the simultaneous surface expression of Ti-T3. T3- cells could not be stimulated by anti-Thy-1 or Ly6 antibodies, and constitution of T3 expression by gene transfer led to reacquisition of the ability to be stimulated by these antibodies.

These studies will enhance our knowledge of which cell membrane molecules are involved in triggering T cells to exert regulatory and effector function, and our understanding of the structural basis for specific T cell responses to antigen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00425-03 LI

PERIOD COVERED

October 1, 1986 to September 31, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Lymphocyte Physiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. M. Chused	Senior Investigator	LI, NIAID
Others:	Y. A. Ishida	Visiting Fellow	LI, NIAID
	E. M. Brown	Biologist	LI, NIAID
	D. R. Greenblatt	Guest Worker	LI, NIAID
	Y. Y. Ji	Guest Worker	LI, NIAID

COOPERATING UNITS *(if any)* W. E. Paul, Chief, LI, NIAID; L. Samelson, Senior Staff Fellow, CBMB, NICHHD; R. Klausner, Chief, CMBM, NICHHD; R. Tsien, Associate Professor, U. C. Berkeley; F. Finkelman, Professor, Dept Med., USUHS.

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NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.9	1.9	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The process of signal transduction across the lymphocyte plasma membrane is under investigation. Novel fluorescent probes of physiologic parameters such as membrane potential, intracellular free ionized calcium, and intracellular pH, in conjunction with the high sensitivity and single cell resolution of flow cytometry, are being utilized.

PHS 6040 (Rev 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00427-03 LI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antigen-Specific and Antigen-Nonspecific Cellular Cytotoxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michail V. Sitkovsky	Visiting Scientist	LI, NIAID
Others:	Hajime Takayama	Visting Fellow	LI, NIAID
	Guido Trenn	Guest Worker	LI, NIAID

COOPERATING UNITS *(if any)*

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NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have attempted to identify proteins and enzymatic activities involved in T cell-receptor (TcR)-triggered cytotoxic T lymphocyte (CTL) activation and effector functions. Several independent complementary approaches were used. A novel functional assay for CTL activation was developed by taking advantage of granule location of an easily detectable enzymatic activity. It is demonstrated that intensity of response (exocytosis of granules) can be manipulated by changing surface density of an immobilized anti-TcR mAb. Using such an assay, we found that TcR-triggered exocytosis is not constitutive and is regulated through cross-linking of TcR by antigen or anti-TcR mAb. The results of experiments utilizing different specific inhibitors and chelators are consistent with a model in which TcR-triggered exocytosis in CTL is mediated by protein kinase C activation, external Ca^{++} translocation through plasma membrane Ca^{++}-channels and modulation of activity of Ca^{++}, calmodulin (CaM)-dependent enzymes. Detailed studies of TcR-triggered exocytosis allowed us to identify an inhibitory biochemical pathway in CTL. We have found that cAMP - raising toxins and drugs as well as cAMP-dependent protein kinase activators inhibit TcR-triggered exocytosis, thus functioning as a possible "OFF" signalling pathway. Work is now in progress to identify surface antigens which are coupled to this inhibitory pathway. It is shown that inhibition of CTL activation by anti-Lyt-2 mAb is not due to the triggering of a biochemical inhibitory pathway. The powerful effects of protein kinase C activators on lymphocyte physiology prompted us to consider them as potential immunomodulating drugs. We have found, that compounds of the Bryostatin family (polycyclic lactone with protein kinase C activating and anti-cancer properties) are not lymphotoxic and can express both immunostimulating and immunosuppressive activities.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00493-01 LI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

B Cell Stimulatory Factor-1 (BSF-1)/Interleukin-4 (IL-4)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	William E. Paul	Chief	LI, NIAID
Others:	J. Ohara	Visiting Scientist	LI, NIAID
	C. Snapper	Medical Staff Fellow	LI, NIAID
	C. Peschel	Visiting Associate	LI, NIAID
	M. Plaut	Guest Researcher	LI, NIAID
	G. Trenn	Guest Researcher	LI, NIAID
	M. Sitkovsky	Visiting Scientist	LI, NIAID

COOPERATING UNITS *(if any)* Oregon Health Sciences University (M. Brown); USUHS (F. Finkelman); Dept Agriculture (J. Urban); NCI (J. Pierce); NCI (A. Kruisbeek); Harvard School of Public Health (L. Glimcher); Harvard Medical School (A. Abbas); WRAIR (M. Meltzer); WRAIR (R. Crawford).

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NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.1	3.1	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

B Cell Stimulatory Factor-1 (BSF-1) is a T cell-derived lymphokine originally defined on the basis of its action as a co-stimulant of B cell division. It has been purified to homogeneity, an N-terminal amino acid sequence determined, a monoclonal anti-BSF-1 antibody has been derived, and BSF-1 cDNA clones have been generated. BSF-1 has multiple actions on cells of hematopoietic lineage. Among these is a striking action in the regulation of IgG1 and IgE production by B cells stimulated in vitro with lipopolysaccharide. The effect appears to be mediated by Ig class switching and is also important in vivo since anti-BSF-1 antibody blocks IgE production by mice infected with Nippostrongylus brasiliensis larvae or inoculated with anti-IgD antibody. Further suggestive evidence for the involvement of BSF-1 in IgE-mediated events is the demonstration that transformed mast cell lines and factor-dependent, non-malignant mast cell lines express mRNA for BSF-1 and supernatant fluids of transformed lines contain BSF-1.

BSF-1 has potent actions on the growth of T cells and on their differentiation to cytotoxic T cells; it causes the induction of cytotoxic potential in macrophages, and enhances expression of class II major histocompatibility complex molecules in such cells. It is a potent co-stimulant of the growth of colonies from various hematopoietic lineage precursors, including myelomonocytic precursors, megakaryocytic precursors, and immature erythroid precursors.

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LABORATORY OF IMMUNOPATHOLOGY
1987 Annual Report
Table of Contents

Z01 AI Project Number		Page
Summary		9-1
00013-24	Immunobiology and Pathogenesis of DNA Virus Infections -- Lewis	9-7
00135-13	Properties of Immunoglobulin Secreting Cells -- Morse	9-8
00138-13	Viruses and Immune Response -- Morse	9-9
00205-07	Abnormalities of T and B Lymphocytes of Autoimmune Mice -- Morse	9-10
00284-06	Characterization of Pathogenic Murine Leukemia Viruses -- Hartley	9-11
00286-06	Studies of Genetic Control of Murine Leukemia Viruses and Virus-Induced Neoplasms -- Hartley	9-12
00465-02	Retrovirus-induced Murine Immunodeficiency Syndrome -- Morse	9-13
00484-01	Mechanisms in Hematopoietic Cell Differentiation -- Holmes	9-14

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SUMMARY REPORT

ANNUAL REPORT OF THE LABORATORY OF IMMUNOPATHOLOGY, NIAID
October 1, 1986 to September 30, 1987

Herbert C. Morse III, M.D.
Chief, Laboratory of Immunopathology

Current studies in the Laboratory of Immunopathology are focused primarily on C-type viruses of the mouse and papovaviruses. The major goal of the retrovirus program is understanding the importance of these viruses to the biology of the mouse, chiefly with regard to transformation of hematopoietic cells and induction of immune suppression. Studies of retroviral pathogenesis are a continuation of long term efforts by the Rowe/Hartley laboratory to elucidate the mechanisms by which endogenous and exogenous virus infections contribute to various neoplasias, to define host genes that influence virus expression and tumor development and to understand the organization of the viral genome and its relation to disease. Advantage is taken of a wide variety of virus stocks that induce specific tumors and mouse strains congenic for ecotropic virus induction loci, loci controlling virus spread in the mouse (Fv-1, Rmcf), and H-2 haplotypes. Over the last few years, the program has increasingly used molecular genetic approaches to complement in vivo pathogenicity tests in studying the contributions of viral LTR sequences to tumor induction (Hartley, Morse, Chattopadhyay) and assessing the effects of oncogenes included in replication-defective viral constructs (Morse, Hartley, Holmes). Although much of the molecular work has been carried out as part of high quality, long term collaborations with others (N. Hopkins, MIT; U. Rapp, NCI), the addition of Dr. Sisir Chattopahdyay has permitted major and direct involvement of LIP in these studies.

The large number and spectrum of tumors developing in virus-infected mice provide a unique resource for studies of hematopoietic differentiation. A panel of antibodies to cell surface antigens has been developed to define, by flow cytometry, the lineage relationships and states of differentiation exhibited by primary tumors and cell lines derived from them (Holmes, Morse). These phenotypic studies are complemented by molecular analyses of immunoglobulin and T cell receptor gene organization and expression of protooncogenes and by histopathologic studies of the tumors by Dr. T. Fredrickson. Particular emphasis is placed on the relation of B cell and myeloid cell development, (Holmes, Morse) the differentiation and function of Ly-1$^+$ B cells and the role of altered myc and raf expression in tumorigenesis (Morse, Holmes). Similar approaches have been utilized to study cellular mechanisms related to development of autoimmune disease in mice homozygous for the nonallelic, autosomal recessive mutations lpr and gld as part of long standing collaborative studies with Dr. W. Davidson (LG, NCI).

The complementing expertise in retrovirology, immunology and histopathology in the Viral Oncology and Virology and Cellular Immunology Sections has been exploited in developing broadly based studies of immune suppression induced by unique murine C-type viruses, termed LP-BM5 MuLV. Although the etiologic viruses differ from the lentiviruses causal in AIDS, studies of inoculated mice have revealed a spectrum of immune defects similar to those in humans infected with HIV. This rapidly expanding program has taken a variety of directions including the biologic and molecular characterization of the viruses (Yetter, Hartley, Chattopadhyay), evaluations of genetic determinants of resistance and susceptibility to disease (Yetter, Morse), identification of target cells for infection, and dissection of cellular interactions contributing to immune suppression (Yetter, Morse). From their inception, these studies have benefitted from outstanding collaborations with Dr. D. Mosier (MBI, LaJolla, CA) and have achieved their current scope through extremely productive interactions with a large number of other scientists.

Studies of human adenoviruses and papovaviruses in the Viral Pathogenesis Section have centered on the biology of adenovirus 2-SV40 hybrids, the parental viruses and several other oncogenic DNA viruses. The viruses are being studied for their transforming activities in vitro and in vivo in several species, with emphasis on defining the characteristics of cells targeted for transformation (Carbone, Lewis) and elucidating cellular mechanisms of the host and the tumor that determine growth or regression of transplanted neoplasms (Lewis, Haddada, Carbone). Biochemical studies, both protein and molecular, of transformed cells and inducing viruses have gained increasing importance over the last several years and a variety of these techniques are now established in the Laboratory. The program has been complemented by long term collaborations with Dr. A. Levine (NICHHD) and Dr. J. Cook (NJH, Denver, CO).

Specific highlights of the last year's work in the Laboratory are as follows:

Requirement for deregulated myc expression in plasmacytomagenesis. Translocations involving the c-myc locus that alter expression of this onc gene are present in over 95% of murine plasmacytomas induced by pristane alone suggesting a central role for aberrant myc expression in the development of this type of tumor. To directly test the importance of myc deregulation to plasmacytomagenesis, BALB/c mice treated with pristane were inoculated with a recombinant retrovirus containing the avian v-myc gene. Infected mice developed plasmacytomas with short latencies. Strikingly, the tumors did not exhibit translocations involving c-myc. Instead, they contained v-myc sequences and expressed the gene at high levels. These results provide the most direct evidence available for a central role for altered *myc* expression in plasmacytomagenesis (Potter, Mushinski, Brust, Babonets, Wiener, Rapp, Morse).

Determination of allelic exclusion by membrane IgM. The possibility that expression of membrane IgM might control allelic exclusion was tested in mice transgenic for an intact human IgM heavy chain gene that can only be expressed at the cell surface. Flow cytometry studies of spleen cells from these mice showed that B cells expressing the human transgene do not express mouse IgM and that the few cells expressing mouse IgM do not express the transgene. Thus, the membrane form of IgM is sufficient to mediate allelic exclusion (Nussenzweig, Holmes, Morse, Leder).

T cells are required for induction of MAIDS. C57BL/6 mice infected with LP-BM5 MuLV rapidly develop lymphadenopathy and profound immunosuppression. The role of T cells in disease was examined by studying nude mice infected with these viruses. We found that infected nude mice did not develop lymphadenopathy or B cell abnormalities even though they replicated the viruses at high levels. These findings indicate that mature T cells are required for the development of MAIDS (Mosier, Yetter, Morse).

T cell receptor/T3 phosphorylation of abnormal lpr and gld T cells. A markedly expanded population of Thy-1$^+$, Ly-2$^-$, Ly-4$^-$ T cells is responsible for the lymphadenopathy of mice homozygous for the *lpr* and *gld* mutations. These cells fail to respond to antigenic stimuli in spite of the fact that they have a normal complement of T cell receptors. To determine if this lack of responsiveness could be due to abnormal signaling through the receptor, we analyzed receptor/T3 phosphorylation patterns. We demonstrated constitutive tyrosine phosphorylation of the T3 p21 protein as characteristic of both *lpr* and *gld* abnormal T cells. This common abnormality extends the parallels between the effects of these distinct mutations and suggests that it may contribute to the autoimmune defects these mice develop (Davidson, Samelson, Klausner, Morse).

Ly-4$^+$ helper T cells are not required for induction of in vivo cytotoxic T lymphocyte (CTL) responses. CTL provide a major line of defense against a variety of intracellular pathogens. Earlier studies of the cellular interactions required for CTL induction *in vitro* indicated that these responses were completely dependent on help provided by Ly-4$^+$ T cells. To study the requirement for Ly-4$^+$ cells during generation of virus-specific CTL responses *in vivo*, mice were depleted of Ly-4$^+$ cells by treatment with anti-Ly-4 monoclonal antibody and then challenged with ectromelia virus. Infected animals survived the infection and developed a CTL response equal to that of intact mice. These results demonstrate a novel mechanism of resistance to infection *in vivo* (Buller, Holmes, Fredrickson, Morse).

Transmissible spongiform encephalomyelopathy in grey tremor (gt) mice. The neurologic disease of mice homozygous for the *gt* mutation resembles that induced by infection of mice with the neurotropic retrovirus, Cas-Br-M, or with the slow virus, scapie. Cell free extracts of gt brains inoculated into NFS/N mice were shown to induce clinical disease due to spongiform encephalopathy. No retroviruses were detected in the brain extracts or tissues of inoculated mice and scrapie-associated fibrils were not detected in brains of affected mice. These results indicate that gt neurologic disease has both genetic and transmissible components and may be associated with a novel unconventional agent (Hoffman, Rowhrer, Bilello, Hartley, Morse).

Specific portions of enhancer sequences in LTRs of Friend and Moloney MuLVs can be identified with target cell specificity. The major determinant of disease specificity of Friend and Moloney MuLVs in NFS mice lies within U3 and is contained within about 200 nucleotides comprising enhancer sequences and a short GC-rich portion of the promoter region. In the enhancer, a core region of exact homology is flanked by segments in which occur the limited nucleotide differences between the two viruses. The 3' segment has stronger specificity for determining Moloney-type T-cell lymphoma or Friend-type erythroleukemia but the 5' segment has some directing effect and promoter sequences may also contribute to specificity. (Hartley, Hopkins, Fredrickson).

Expression of MCF MuLV in thymus is strongly correlated with ability of a virus to induce T-cell lymphoma. Virus recovery assays of lymphoid tissues of mice early after inoculation with Moloney, Friend, and recombinant constructs involving the LTR region of these viral genomes have shown that although minor differences can be detected in number of thymus cells replicating ecotropic virus the major correlate of disease has proved to be the generation of MCF virus in thymus, as a predictor of T-cell lymphoma. (Hartley, Hopkins).

Immunosuppressive, lymphoproliferative disease of mice ("MAIDS") appears to require two replication competent MuLVs. Attempts to clone biologically the components of LP-BM5 virus have confirmed the regular association of B-tropic ecotropic and B-tropic MCF MuLVs in induction of disease. Pathogenicity testing of cloned ecotropic and MCF isolates indicates that infection of mice with virus of each type is generally required for disease induction, but a highly active cloned MCF virus has not yet been identified. Biological and molecular characterization of ecotropic and MCF isolates from various tissue culture and mouse tissue sources suggest that biological and molecular variants are common, at least following inoculation of partially resistant strains. (Hartley, Yetter, Chattopadhyay).

Strain distribution of sensitivity to LP-BM5 disease is complex. The viruses of the LP-BM5 complex are B-tropic but only a few mouse strains of the Fv-1^b genotype (i.e., C57BL strains and I/St) show rapid induction of progressive lymphoproliferative disease and death within about 6 mos. In addition certain Fv-1^n strains, notably C57L, P/N, BDP, and AKR, develop disease, albeit with delayed onset and lower frequency. These findings, together with indications of modification of the viruses following infection of partially resistant mouse strains, suggest that both host and virus genetic factors are of importance. (Yetter, Hartley, Chattopadhyay, Morse).

Immunological surveillance against DNA virus transformed cells: correlations between natural killer cell cytolytic competence and tumor susceptibility of athymic rodents. Nude mouse natural killer cells that have the capacity to lyse tumor cell targets of murine origin are defective in their capacity to lyse xenogeneic Ad2 transformed hamster cells. In marked contrast, natural killer cells from nude rats lysed xenogeneic Ad2 transformed hamster cells very efficiently, and nude rats were highly resistant to tumor induction when injected with those Ad2 transformed hamster cells unless their natural killer cell responses were compromised. These results indicate that thymus-dependent, cytotoxic T lymphocyte-mediated host cellular immune responses are not essential for the rejection of cells transformed by Ad2 and suggest that natural killer cell mediated, immunologically nonspecific, host cellular immune responses are sufficient to reject cells transformed by nononcogenic Ad serotypes. (Cook and Lewis).

Expression of class I major histocompatibility antigens by adenovirus type 2 and 12 and SV40 transformed Syrian hamster cells. Ad2 transformed hamster cells may express either low, high, or intermediate levels of class I MHC antigens. Like rat and mouse cells, Ad12 transformed hamster cells uniformly express low levels of class I MHC antigen. SV40 transformed cells uniformly express high levels of class I MHC antigen. When we compared these results with the tumor inducing capacity of these cells, we found that nontumorigenic Ad2 transformed hamster cells expressed low levels of class I MHC antigens while Ad2 transformed cells adapted to produce tumors in syngeneic adult hamster expressed high levels of class I MHC antigens. SV40 transformed cells that were highly tumorigenic in both syngeneic and allogeneic adult hamsters uniformly expressed high levels of class I MHC antigens. Levels of class I MHC mRNA generally correlated with the level of class I MHC protein produced. These results imply that if expression of low levels of class I MHC antigens played a role in the tumorigenicity of Ad12 transformed cells, this mechanism is most likely restricted to cells transformed by this virus and does not play a role in tumor induction by SV40 transformed cells or the lack of tumorigenicity of Ad2 or in the mechanism by which Ad2 transformed cells can be adapted to produce tumors in adult hamsters. (Haddada, Lewis, Sogn, Coligin, Levine).

Levels of bovine papillomavirus RNA and protein expression correlate with variations in the tumorigenic phenotype of hamster cells. Of the 3 independent lines of BPV-1 transformed hamster cells that we have produced, 2 lines were highly tumorigenic in nude mice and syngenic as well as allogeneic hamsters; whereas one line failed to produce tumors in any of these animals. Cells from each of these lines contained similar copy numbers of the BPV-1 genome. However the nontumorigenic BPV cell line contained very small quantities of BPV-1 E5 (early 5) transforming protein and very low levels of BPV-specific RNA. The tumor inducing capacity of each of these 3 cell lines seemed to correlate directly with the amount of detectable E5 protein produced. Besides providing an interesting model to assess the possible association between expression of a specific viral- induced early proteins with tumorigenicity, the nontumorigenic BPV cells provide a unique resource to assess those virus-induced and host defense-determined factors that result in the absence of tumorigeneicity by an otherwise immortalized and neoplasticially transformed cells that contain a large burden of integrated viral genome. (Zhang, Lewis, Wade-Glass, Schlegel).

Administrative Changes. Dr. Sisir Chattopadhyay joined the Laboratory in an expert position. Dr. Kevin Holmes was appointed Acting Head of the Flow Cytometry Section of the biologic Resources Branch, OSD, but retains a scientific alliance with the Laboratory.

Honors and Awards

Public Health Service Commendation Award - Herbert C. Morse III.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00013-24
LIP

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Immunobiology and Pathogenesis of DNA Virus Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI :	A. M. Lewis, Jr.	Hd, Viral Pathogenesis Section	LIP, NIAID
Others:	M. Carbone	Visiting Fellow	LIP, NIAID
	J. A. Sogn	Senior Investigator	LIG, NIAID
	J. Coligan	Senior Investigator	LIG, NIAID
	A. S. Levine	Scientific Director	OSD, NICHD
	C. T. Patch	Senior Investigator	OSD, NICHD
	R. Schlegel	Senior Staff Fellow	PB, DCT, NCI

COOPERATING UNITS (if any)
National Jewis Center for Immunology and Respiratory Medicine, Denver, CO (J. L. Cook).

LAB/BRANCH
Laboratory of Immunopathology

SECTION
Viral Pathogenesis Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 4.0	PROFESSIONAL: 1.0	OTHER: 3.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☑ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Current thinking about the mechanism of carcinogenesis by human adenoviruses revolves around the capacity of different adenovirus serotypes to induce in infected or transformed cells the ability to succumb to or resist destruction by the specific (mediated by cytotoxic lymphocyutes) or nonspecific (mediated by NK cells and macrophages) components of the cellular immune system. Last year we identified the E1A oncogene of the nononcogenic Ad2 and Ad5 as the inducer of susceptibility to NK lysis and found that certain Ad2 and Ad5 transformed mouse and hamster cells can express low levels of class I MHC antigens that are comparable to the low levels present on Ad12 transformed cells. These Ad2 and Ad5 transformed cells were unable to produce tumors in syngeneic adult hamsters and mice; Ad12 transformed cells from these species were highly tumorigenic in syngeneic adult animals. This year we have shown that the E1A oncogene of Ad2 and Ad5 induces susceptibility to NK cell lysis during acute infection and have found that Ad12 transformed mouse cells that have low levels of class I MHC antigen on their surface are highly immunogeneic in bioassays for Ad12 transplantation antigen and fail to produce tumors in allogenic mice. Also, we have found that nude mice but not nude rats possess a defect in the capacity of their NK cells to lyse xenogenic cells transformed by nononcogenic Ad. These data are consistent with the conclusion that nononcogenic human Ad induce high levels of susceptibility to lysis by NK cells and macrophages during cell transformation in vivo or in vitro and that neoplastic cells resulting from infection with these Ad are eliminated before they become clinically significant tumors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00135-13 LIP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Properties of Immunoglobulin Secreting Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI :	H. C. Morse III	Chief	LIP, NIAID
Others:	W. F. Davidson	Visiting Scientist	LG, NCI
	J. W. Hartley	Senior Investigator	LIP, NIAID
	T. N. Fredrickson	Research Microbiologist	LIP, NIAID
	K. L. Holmes	Sr. Staff Fellow	LIP, NIAID
	S. P. Klinken	Visiting Fellow	LIP, NIAID
	M. A. Principato	Guest Researcher	LIP, NIAID
	U. R. Rapp	Senior Investigator	LVC, NCI

COOPERATING UNITS (if any)

DNAX Research Institute, Palo Alto, CA (R.L. Coffman); Sloan-Kettering Cancer Center, New York, NY (U.R. Hammerling)

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Continuous cell cultures obtained by in vitro transformation of bone marrow cells with retroviruses containing ras or raf and myc were found to exhibit characteristics of both B cell lineage and myeloid cells. Analyses of cloned derivatives of these cultures showed that lines with phenotypes of mature B cells or mature macrophages could be isolated but that these lines were clonally related. These results indicate that some B cells and myeloid cells can develop from a common progenitor.

The role of altered myc expression in plasmacytoma development was studied by inoculating pristane-primed BALB/c mice with a retrovirus expressing avian v-myc. Over 25% of infected mice developed plasmacytomas that lacked chromosomal translocations affecting the c-myc locus. Instead, the tumors contained and expressed high levels of v-myc. These results provide the most direct evidence to date for a central role for altered myc expression in plasmacytomagenesis.

Mice transgenic for the membrane form of human IgM were studied for expression of murine and human IgM on B cells. By flow cytometry, B cells but not T cells or macrophages were shown to express the transgene. In addition, it was found that B cells expressed either human IgM or mouse IgM indicating that the membrane form of IgM can signal allelic exclusion.

PHS 6040 (Rev. 1/84)

GPO 91 13

Other Investigators:

M. Potter	Chief	LG, NCI
J. F. Mushinski	Investigator	LG, NCI
J. H. Pierce	Investigator	LCMB, NCI
P. Leder	Investigator	Dept. Genetics, Harvard
M. Mussenzweig	Investigator	Dept. Genetics, Harvard

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00138-13
LIP

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Viruses and Immune Response

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI :	H. C. Morse III	Chief	LIP, NIAID
Others:	J. W. Hartley	Senior Investigator	LIP, NIAID
	K. L. Holmes	Senior Staff Fellow	LIP, NIAID
	T. N. Fredrickson	Research Microbiologist	LIP, NIAID
	R. M. L. Buller	Senior Staff Fellow	LVD, NIAID
	A. Singer	Senior Investigator	I, NCI

COOPERATING UNITS *(if any)*

V.A. Research Center, Baltimore, MD (P. Hoffman)

LAB/BRANCH
Laboratory of Immunopathology

SECTION
Virology and Cellular Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mice homozygous for the grey tremor (gt) mutation develop an accerlated spongiform encephalopathy similar to that exhibited by NFS/N mice infected with the wild mouse ecotropic virus, Cas-Br-M. Newborn NFS/N mice inoculated with cell-free extracts of gt brains developed tremor and paralysis associated with spongiform changes in brain indicating that the disease was transmissable. Murine retroviruses and scrapie-associated fibrils (SAF) were not detected in gt or recipient brains suggesting that the disease is caused by an unconventional agent different from scrapie-like agents.

Resistance to ectromelia virus infection in vivo was shown to develop normally in mice depleted of L3T4$^+$ cells. Depleted mice had virus-specific cytotoxic T lymphocyte (CTL) responses equal to those of normal mice indicating that help provided by L3T4$^+$ T cells is not required for induction of CTL responses to modified self determinants in vivo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00205-07 LIP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Abnormalities of T and B Lymphocytes of Autoimmune Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI :	H. C. Morse III	Chief	LIP, NIAID
Others:	W. F. Davidson	Visiting Scientist	LG, NCI
	L. Samelson	Investigator	CBMB, NICHHD
	R. Klausner	Chief	CBMB, NICHHD
	M. Seldin	Investigator	ARB, NIAMSSD
	A. D. Steinberg	Investigator	ARB, NIAMSSD

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
1.0	0.75	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mice homozygous for either of the autosomal recessive mutations, lpr or gld, have a large number of abnormalities in common including marked expansion of an unusual subset of T cells that are Thy-1^+, Ly-1^+, Ly-2^-, Ly-4^- and $B220^+$. In spite of the fact that these cells express T cell receptors for antigen, they fail to respond to stimulation with alloantigens. Biochemical analyses of the T3/T cell receptor complex showed that all components were present as on normal T cells but they exhibited anomalous patterns of phosphorylation. Normal T cells stimulated with antigen or lectin exhibit phosphorylation of p21 on tyrosine and gp21 on serine. By comparison, for lpr and gld T cells the p21 component of T3 was phosphorylated in the absence of stimulation whereas the gp21 component of T3 was not.

Studies devoted to cloning the gld gene have been initiated with the characterization of molecular clones located near the mutant gene on chromosome 1. Analyses of Ly-5 among inbred and wild strains of mice revealed 5 new alleles defined by serology and restriction fragment length polymorphisms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00284-06 LIP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Pathogenic Murine Leukemia Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI :	J. W. Hartley	Hd, Viral Oncology Section	LIP, NIAID
Others:	H. C. Morse III	Chief	LIP, NIAID
	T. N. Fredrickson	Research Microbiologist	LIP, NIAID
	R. A. Yetter	Guest Researcher	LIP, NIAID
	K. L. Holmes	Senior Staff Fellow	LIP, NIAID
	S. K. Chattopadhyay	Expert	LIP, NIAID
	C. Kozak	Senior Investigator	LMM, NIAID
	R. Repaske	Senior Investigator	LMM, NIAID
	R. O'Neill	Investigator	NINCDS

COOPERATING UNITS (if any)

Massachusetts Inst. of Technology, Cambridge, MA (N. Hopkins); Johns Hopkins, Baltimore, MD (S. Staal); Georgetown Univ., Washington, DC (B. Baroudi); Univ. of Massachusetts, Worcester, MA (C. Holland)

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 4.0	PROFESSIONAL: 2.3	OTHER: 1.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is concerned with isolation and biological and molecular characterization of C-type murine leukemia viruses (MuLVs), with emphasis on the replication competent non-oncogene containing agents. A major objective is the delineation of the molecular genetics of MuLV-related pathogenesis in mice of leukemias and lymphomas, and non-neoplastic changes such as lymphoproliferation, immunosuppression, or neurologic disease. Attempts to identify viral genes which determine phenotypes such as type of disease use recombinant virus genomes constructed from molecular clones of viruses with differing properties. Mice of selected inbred strains are inoculated with virus preparations which represent biologically or molecularly cloned stocks of parental, recombinant, or site-specific mutant genomes. Replication of virus is measured and mice are examined for gross and microscopic pathology, and tumors may be examined by flow cytometry, for recovery of virus, or molecularly for host gene alterations such as new proviral integrations or gene rearrangements. Studies with Moloney and Friend MuLVs, which induce in NFS mice almost exclusively T-cell lymphoblastic lymphoma or erythroleukemia, respectively, have established that the major determinant of disease specificity lies within that portion of the LTR comprising direct repeat sequences identified with virus transcriptional enhancer signals, one element of the enhancer having a predominant effect. Chimeric genomes are also being tested in an attempt to clarify the mechanism of lymphoma induction by an unusual MCF virus, NS6 MCF. In a third system, the LP-BM5 lymphoproliferative and immunosuppression syndrome, isolation and characterization of the components of disease-inducing virus stocks are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00286-06 LIP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Genetic Control of Murine Leukemia Viruses and Virus-Induced Neoplasms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI :	J. W. Hartley	Hd, Viral Oncology Section	LIP, NIAID
Others:	T. N. Fredrickson	Research Microbiologist	LIP, NIAID
	R. A. Yetter	Guest Researcher	LIP, NIAID
	S. P. Klinken	Visiting Fellow	LIP, NIAID
	M. Principato	Guest Researcher	LIP, NIAID

COOPERATING UNITS (if any)

USC School of Medicine (P. Pattengale); U. of Texas System Cancer Center (F. Becker).

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	1.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Genetic factors contributed by both host and virus interact to determine the effects of murine leukemia virus (MuLV) infection of mice. Host genes which influence MuLV-related hematopoietic system disease have been identified and chromosomally mapped; these include MuLV ecotropic virus induction loci, which represent integrated proviral sequences, and genes controlling infection and spread of virus, such as Fv-1, determining sensitivity to infection by N- or B-tropic MuLVs, and Rmcf, which affects replication and generation of MCF viruses. In order to study gene effects isolated from other host genetic differences, a number of strains of NFS mice congenic for different virus induction loci (v-congenics) and for the MCF MuLV resistance allele Rmcf^r have been established. Profiles of spontaneous disease have been established for the v-congenics and those with high ecotropic MuLV expression are used in pathogenicity assays of MCF viruses, which may require helper virus to express oncogenic activity. Their use has led to the isolation of 2 new ras-containing acute transforming viruses and an unusual variant of LP-BM5 MCF virus has been revealed. Studies of host factors influencing MuLV related disease utilize NFS-Rmcf^r congenics for analysis of LTR-region recombinants of Moloney and Friend MuLV, and a variety of inbred strains are used for investigation of the strain distribution of sensitivity to LP-BM5 disease. In the latter case, host genes which are known to affect MuLV spread appear to have reduced activity on certain genetic backgrounds.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00465-02 LIP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Retrovirus-induced Murine Immunodeficiency Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI :	H. C. Morse III	Chief	LIP, NIAID
Others:	R. A. Yetter	Guest Researcher	LIP, NIAID
	J. W. Hartley	Senior Investigator	LIP, NIAID
	C. Shearer	Investigator	I, NCI
	J. R. Wunderlich	Investigator	I, NCI
	K. L. Holmes	Senior Staff Fellow	LIP, NIAID
	S. P. Klinken	Visiting Fellow	LIP, NIAID
	S. K.Chattopadhyay	Expert	LIP, NIAID

COOPERATING UNITS *(if any)*

Medical Biology Institute, LaJolla, CA (D. Mosier); Johns Hopkins Medical School, Baltimore, MD (P. Pitha).

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mice infected with LP-BM5 murine leukemia viruses (MuLV) develop a syndrome of lymphadenopathy and profound immunosuppression with many immunologic features in common with AIDS. Development of this disease is completely dependent on the activity of T cells since infected nude mice do not develop B cell abnormalities. In addition, it has been found that development of disease is inhibited in mice depleted of Ly-4^+ T cells before infection and that many aspects of disease are reversed in animals depleted of Ly-4^+ cells after infection. The late stages of LP-BM-5-induced disease are associated with enhanced susceptibility to infection and the development of B cell lymphomas; C57BL/6 mice infected with LP-BM5 MuLV become susceptible to lethal infection with ectromelia virus and oligoclonal or monoclonal proliferations of B cells are detected in lymphoid tissues of almost all moribund mice.

Other Investigators:

R. M. L. Buller	Senior Staff Fellow	LVD, NIAID
T. N. Fredrickson	Research Microbiologist	LIP, NIAID
W. E. Paul	Chief	LI, NIAID

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00484-01 LIP

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanisms in Hematopoietic Cell Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI :	K.L. Holmes	Hd, Flow Cytometry Section	BRB, NIAID
Others:	H.C. Morse, III	Chief	LIP, NIAID
	Hartley, J.W.	Hd, Viral Oncology Section	LIP, NIAID
	Davidson, W.F.	Senior Investigator	LG, NCI
	Pierce, J.H.	Senior Investigator	LCMB, NCI
	Potter, M.P.	Chief	LG, NCI
	Yetter, R.A.	Senior Investigator	LIP, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
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SECTION
Cellular Immunology Section

INSTITUTE AND LOCATION
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The use of neoplasms as model systems for hematopoietic cell differentiation has been well established. We have studied a wide variety of in vitro and in vivo derived tumors induced with the ecotropic MuLV, Cas-Br-M, with retroviruses containing the oncogenes ras, bas, fes, src and abl, or with chemical agents. These studies have been complimented with experiments involving transfer of normal cells into mice with severe combined immunodeficiency disease (SCID). We have found that, contrary to current views of hematopoietic cell development, the B cell and the myeloid lineages may share a common progenitor cell and that this cell may be identified by the expression of Ly-17 (Fc gamma receptor) and Mac-1, an antigen thought to be restricted to the myeloid lineage. In addition, studies of T cell lymphomas induced with the Cas-Br-M MCF virus, NS-6, and the SCID mice experiments suggest that Mac-1 may also be present on T cell progenitors.

We have extensively utilized two very different model systems to study the changes in bone marrow hematopoiesis associated with perturbations in the periphery. Inoculation of mice with the anemia-inducing drug phenylhydrazine was shown to stimulate B, T and myeloid cell differentiation in addition to the production of erythroid progenitors. In contrast, infection with the LP-BM5 immunodeficiency-inducing virus induces profound changes in pre-B and B cells but does not effect the development of other lineages in the bone marrow.

Production of hybridomas against a tumor (BXH-2) that has stem cell characteristics has yielded two monoclonal antibodies that recognize a small population of bone marrow, but not spleen and a subpopulation of SCID spleen.

Project Number Z01 AI 00484-01 LIP

Others:	Klinken, S.P.	Visiting Fellow	LIP, NIAID
	Principato, M.A.	Investigator	LIP, NIAID
	Langdon, W.Y.	Visiting Fellow	LIP, NIAID
	Bauer, S.	Investigator	LG, NCI
	Fredrickson, T.N.	Professor	Univ.of Connecticut

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LABORATORY OF IMMUNOREGULATION
1987 Annual Report
Table of Contents

Z01-AI Project Number		Page
Summary		10-1
00210-07	Immunoregulation of Human Lymphocyte Function in Normal and Disease States - Fauci	10-17
00212-07	Study of the Immunopathogenic Features of Immune-Mediated Diseases - Fauci	10–18
00213-07	Clinical, Immunopathogenic, and Therapeutic Studies in the Spectrum of Vasculitis - Fauci	10–19
00390-04	Studies of the Acquired Immunodeficiency Syndrome - Lane	10–20
00431-03	The Molecular Biologic Approach to the Immune System - Siebenlist	10–21
00361-05	International Studies of the Acquired Immunodeficiency Syndrome - Quinn	10–22
00358-05	Immunopathogenesis of Chlamydia trachomatis Infection - Quinn	10–23

Summary Report
Laboratory of Immunoregulation
October 1, 1986 through September 30, 1987

Anthony S. Fauci, M.D.
Chief, Laboratory of Immunoregulation, NIAID

Studies of the Human B Cell Cycle: Analysis of B Lymphocyte Activation Pathways

Over the past several years, we have established and employed a model system for the dissection of the events involved in the driving of human B cells from the resting state through proliferation and ultimately to terminal differentiation and antibody secretion. We have delineated the necessary and sufficient signals required to activate a resting B cell whereby it becomes responsive to a variety of growth and differentiation factors. The more classic model of B cell activation triggers B cells by crosslinking their surface membrane immunoglobulin (sIg) molecules either by antigen, anti-Ig antibody or Staphylococcus aureus Cowan I (SAC). Last year we described an activation factor produced by a T4 lymphoma line (PEER; BCAF) which can activate resting B cells without crosslinking sIg. This finding has important implications in understanding the mechanisms of polyclonal activation of B cells and amplification of the response of the B cell repertoire in diseases characterized by hyperactivity of B cell function. We are currently purifying the factor for the purpose of cloning its gene and examining its receptor. We have compared the kinetics of the activation process of BCAF and anti-mu. Following activation of resting B cells with BCAF, there is a much slower rise in intracellular calcium which occurs in approximately 25% of cells and is sustained for a much longer period of time than with activation due to anti-mu. In addition, SAC and anti-mu clearly induce the production of both IP3 and diacylglycerol (DAG), while BCAF induces DAG but very little IP3. These findings suggest different activation pathways for BCAF and anti-mu. (Ambrus, Young, Fauci, LIR/NIAID)

Studies of the Human B Cell Cycle: The Identification, Purification, and Characterization of Human B Cell Growth and Differentiation Factors and Their Cellular Receptors

Three years ago, we identified and isolated a high molecular weight B cell growth factor (HMW-BCGF) produced by a human B cell lymphoma line as well as by a T cell lymphoma line. The factor had a MW of approximately 60 Kd, and it preferentially enhanced the proliferation of normal human B cells which were already in the activated state and hence it provided a second signal for proliferation later on in the sequential steps of the B cell cycle. Last year we developed a monoclonal antibody (BCGF/1/C2) against the factor and also identified and characterized its receptor which is recognized by a monoclonal antibody (BA5). Over the past year we have used a number of approaches aimed at cloning the genes for the HMW-BCGF. We have upscaled our purification procedures and have generated 50 ug of purified factor. This is currently being sequenced and will be cleaved into peptides if N-terminal blocking is still a problem as it has been over the past year. Our previous attempts at cloning by using a cDNA library of activated Namalva cells (source of HMW-BCGF) expressed in lambda phage and screened with monoclonal and polyclonal anti-HMW-BCGF have been unsuccessful and so we are currently utilizing a rabbit reticulocyte lysate expression system and activated Namalva mRNA. We have recently shown that we can produce a 50Kd protein with BCGF activity which is recognized by BCGF/1/C2.

We have previously demonstrated that the receptor for HMW-BCGF is a 90 Kd heterodimer with a 48 Kd associated protein. A monoclonal antibody (BA5) recognizes the receptor which is expressed at high levels on the Epstein-Barr virus (EBV)-transformed B cell line MB. We have employed the MB line to purify receptor protein. Intact receptor was N-terminally blocked, and therefore, we have prepared internally labelled CNBr peptides from the intact protein and have repurified them on high performance liquid chromatography (HPLC). We are currently sequencing several of the peptides to obtain sufficient sequence for cloning.

In other studies, we are analyzing the complete structure of the HMW-BCGF receptor. We have demonstrated that following SAC-activation of small elutriated B cells, 30 Kd, 48 Kd, and 50 Kd precursor proteins recognized by BA5 and by a polyclonal heterologous antiserum (S86) appear in the cytosol within 1 hour while the 90 Kd receptor can be found in the cytosol by 4-8 hours. Expression of the receptor at the surface membrane occurs between 8-12 hours and does not peak until 16-24 hours. As we more precisely define the assembly and expression of the receptor on normal B cells, we will explore its expression and function in diseases characterized by abnormalities of B cell function such as systemic lupus erythematosus (SLE), common variable immunodeficiency (CVI), and various B cell tumors.

We have previously demonstrated secretion of B cell differentiation factor (BCDF) by a T4+ T cell clone (YA2) that was a potent inducer of B cell differentiation. Over the past year, using an HTLV-I transformed clone of YA2 (TYA2) we obtained large quantities of BCDF for purification. Purified BCDF was radiolabelled with ^{125}I and its binding characteristics were examined. Labeled BCDF bound to BCDF responsive cells such as SAC-activated B cells, SKW 6.4 cells, and CESS cells, but did not bind to BCDF non-responsive cells such as resting B cells, resting T cells, and phytohemagglutinin (PHA)-activated T cells. Binding to SAC-activated B cells was competetively inhibited by cold BCDF, but not by interleukin (IL)-2. We are currently conducting studies aimed at identifying and characterizing the cellular receptor for BCDF and at cloning the genes for the factor and its receptor as described above for HMW-BCGF and its receptor.

Last year, we had previously described a T8+ T cell clone (YD2) that markedly suppressed B cell differentiation driven by BCDF or by pokeweed mitogen (PWM) in a T cell dependent system. Over this past year we characterized the supernatant of YD2 and demonstrated that the suppressive factor in the supernatant was PGE^2 It co-eluted with PGE$_2$ on HPLC, it was removed by anti-PGE$_2$, was indistinguishable from PGE$_2$ on radioimmunoassay and identical suppressive activity was seen with purified PGE$_2$. Modulation of B cell responses by a T cell that secretes PGE$_2$ may reflect one of the potential mechanisms whereby the inflammatory process may be involved in the regulation of B cell function. (Ambrus, Goldstein, Young, Siebenlist, Bressler, Fauci, LIR/NIAID; Lewis, Austen, Harvard)

The Structural and Functional Analysis of the Human IL-2 Receptor

It has been well established that there exists a high affinity and a low affinity form of the IL-2 receptor on T cells. Recently, W.C. Greene and others have demonstrated the existence of a second IL-2 binding protein which is approximately 70 Kd (p70) in size and lacks reactivity with the anti-Tac monoclonal antibody which has been used for years to define the human IL-2 receptor. In collaboration with Dr. Greene, we have recently demonstrated that p70 binds IL-2 with intermediate affinity and appears to be necessary for high affinity receptor formation. Chemical crosslinking of ^{125}I-IL-2 which was bound to high affinity IL-2 receptors produced labelling of both the p70 protein and the Tac antigen, while crosslinking to an HTLV-I infected T cell line which expresses only low affinity receptors labels only the Tac antigen. Crosslinking to MLA-144, a gibbon ape T cell leukemia line which binds IL-2 but which is Tac negative, labeled only the p70 protein. The affinity of the IL-2 binding sites on the MLA-144 line was shown to be between the well established high affinity (10 pM) and low affinity (5 nM) sites. Finally, transfection of the Tac cDNA into the MLA-144 line resulted in reconstitution of high affinity receptor expression. These high affinity sites involved the Tac antigen since anti-Tac antibody blocked the high affinity component of the binding. Taken together, these findings suggest that the high affinity human IL-2 receptor may correspond to a membrane complex composed of p70 and Tac.

We reported last year that high concentrations of IL-2 could induce the proliferation of resting T cells. Since these effects occurred in the absence of detectable Tac antigen expression, the possibility existed that the IL-2 might be reacting initially with the p70 protein. Over the past year, we demonstrated by chemical crosslinking studies that the p70 protein was expressed on resting T cells and bound IL-2 with an intermediate affinity which may account for the high concentrations of IL-2 needed to induce proliferation of resting T cells. Furthermore, the early biological consequences of IL-2 binding to the p70 protein were studied at the molecular level. Northern blot analysis revealed the rapid induction of c-myc, c-myb, and Tac mRNA following stimulation of resting T cells with high concentrations of IL-2. Anti-Tac did not inhibit IL-2 induced expression of these genes suggesting that the p70 protein rather than the Tac antigen or the high affinity IL-2 receptor complex mediated this signal. However, in contrast to these early events, the anti-Tac antibody significantly inhibited IL-2 induced T cell proliferation. This finding implicates the high affinity form of the IL-2 receptor in the proliferative response of the IL-2 activated T cells. Thus, these data support a two step model for the induction of resting T cell proliferation by high doses of IL-2 involving the initial generation of an activation or "competence" signal through the p70 protein and a subsequent proliferation or "progression" signal through the high affinity form of the receptor.

Previous studies have demonstrated that the cytolytic activity in peripheral blood lymphocytes and in purified natural killer cells can be markedly amplified in vitro by the addition of IL-2. The effect required high concentrations of IL-2 and was independent of the expression of Tac antigen. We have recently shown that the addition of high concentrations of recombinant IL-2 to enriched NK cells resulted in rapid augmentation of cytolytic activity with a more delayed increase in cellular proliferation. Anti-Tac effectively blocked the IL-2 induced proliferation, but failed to alter the enhancement of cytotoxicity. Chemical crosslinking demonstrated the presence of the p70 protein on the purified NK in the absence of Tac antigen. There were 2300 p70 binding sites per cell with an intermediate binding affinity. Analysis of NK cytoplasmic RNA isolated at various time points following IL-2 stimulation revealed the rapid induction of c-myb and Tac gene expression which was not inhibited by anti-Tac antibody. These findings suggest that IL-2 binding to the p70 receptor which is constitutively expressed on the surface of NK cells may mediate the development of increased cytolytic activity.

Two years ago we reported that IL-2 directly activated B cells to proliferate and differentiate to Ig secretion in the apparent absence of other activation signals. Since the stimulatory effects of IL-2 occurred in the absence of detectable Tac+ cells, we investigated the role of p70 in this activation process. By cell fractionation and crosslinking studies with radiolabeled IL-2, we demonstrated the expression of approximately 80 high affinity sites (p70 plus Tac) on large B cells with no detectable receptors on small B cells. These data suggest that the direct activation of B cells occurs either via the low numbers of high affinity receptors that we have demonstrated or via levels of p70 which are not detectable in our binding and crosslinking assays. We did, however, demonstrate the p70 protein on the Tac negative SKW 6.4 B cell line which responds to high concentrations of IL-2 suggesting that under certain circumstances B cells may be able to express p70 in the absence of Tac. (Kehrl, Fauci, LIR/NIAID; Le thi Bich-Thuy, NCI; Dukovich, Greene, Duke Univiversity; Katz, Georgetown Univiversity)

Regulation of Human B Cell Function by Soluble Factors Other Than the Classical BCGFs and BCDFs

Last year we demonstrated that transforming growth factor-beta (TGF-beta) suppresses IL-2 and BCGF-dependent B cell proliferation, suppresses factor-dependent B cell Ig secretion and is present in supernatants of activated B cell cultures. Over this past year, we have demonstrated that activated B cells express high affinity TGF-beta receptors. We have recently explained the effects of TGF-beta on B cell activation and have shown that phorbol myristic acetate (PMA) and calcium ionophore (ionomycin)-induced B cell prolfieration were totally inhibited although the inhibition was partially reversed by increasing the concentration of ionomycin. These studies sugggest that TGF-beta may be inhibiting c-kinase activation or a calcium-dependent step in B cell activation.

We have recently demonstrated that the monocyte product tumor necrosis factor (TNF)-alpha has significant BCGF activity when assayed on SAC-activated B cells. Using iodinated recombinant TNF-alpha, we performed binding studies on unactivated tonsillar B cells and found low to detectable expression of TNF-alpha receptors. However, after a 2-day activation with SAC, these cells expressed approximately 6,000 receptors per cell with an affinity of 200 pM. Neither IL-1, IL-2, nor IFN-gamma competitively inhibited the binding of radiolabeled TNF-alpha to activated B cells. These studies support a role for TNF-alpha in the regulation of B cell growth.

Since lymphotoxin (LT) is a structurally and functionally related protein to TNF-alpha and a product of activated lymphocytes, we examined the potential role of LT as a B cell growth factor. We found that an antibody to LT removed 70% of the BCGF activity from a supernatant of PHA-activated T cells assayed on SAC-activated B cells. This was not due to a direct toxic effect of the antibody since the antibody did not impair the increase in DNA synthesis induced by recombinant IL-2. Furthermore, the full proliferative response to the T cell supernatant could be restored by adding an excess of recombinant LT. In addition, competitive binding assays confirmed the presence of LT in a partially purified BCGF preparation and Northern blot analysis demonstrated the rapid induction of LT gene expression in PHA-activated T cells. LT may well serve an important role in amplifying the B cell responses in vivo.

We have continued our studies on the role of complement components in human B cell physiology. We have previously demonstrated that the Bb fragment of Factor B enhanced the proliferation of human B cells while Ba had a potent suppressive effect. Recently, we have demonstrated by Western blot analysis an antigenic similarity between HMW-BCGF and Bb. In addition, Bb appears to bind to activated human B cells via the HMW-BCGF receptor. Finally, we have continued our studies on the effect of Clq on B cell function. We have demonstrated that Clq binds to both large and small lymphocytes, does not affect activation or proliferation, and does enhance predominantly IgM production by normal SAC-activated B cells. (Kehrl, Ambrus, Young, Taylor, Fauci, LIR/NIAID; Roberts, Sporn, NCI; Tenner, American Red Cross; Brown, Peters, Washington University; Alvarez-Mon, Madrid, Spain; Miller, University of Illinois)

Clinical Correlates of Studies of Human B Cell Function

We have extrapolated from our studies of the human B cell cycle to the study of immunopathogenic mechanisms of certain diseases characterized by abnormalities of B cell function. B cells from patients with common variable immunodeficiency (CVI) were studied and were shown to respond normally to LMW-BCGF, but abnormally or not at all to HMW-BCGF despite the ability to express receptors for HMW-BCGF. The abnormality was shown to be compatible with a post signal transduction defect. Indeed, treatment of a CVI patient with a nonsteroidal anti-inflammatory agent resulted in normalization of this in vitro defect, suggesting that overproduction of an arachidonic acid metabolite such as PGE or one of the leukotrienes may be playing an inhibitory role in the normal signal transduction occurring with HMW-BCGF.

It is a common clinical observation that certain patients with CVI have allergic reactions (rhinitis, wheezing, skin rash, anaphylaxis) to defined antigens (dust, ragweed, penicillin), but do not have IgE. This suggested an alternative mast cell degranulating mechanism. Indeed, we demonstrated that mononuclear cells from certain CVI patients often proliferated to the involved antigen and consistently released a factor into the supernatant which could induce histamine release from basophils. We are currently biochemically analyzing this factor and attempting to determine its mechanism of effect on basophil histamine release. (Ambrus, Fauci, LIR/NIAID; Buckley, Duke University)

Epidemiologic, Virologic, Molecular Biologic, Immunologic, Clinical, and Therapeutic Studies in the Acquired Immunodeficiency Syndrome (AIDS)

Over the past 5 1/2 years, the LIR has been intensively involved in the study of multiple aspects of AIDS with particular emphasis on the delineation of the immunopathogenic mechanisms of infection with the human immunodeficiency virus (HIV) and on the design and implementation of therapeutic protocols in a wide range of individuals with HIV infection.

We have continued our study initiatied 2 years ago of over 1000 employees of the NIH Clinical Center who are being monitored every 6 months for antibody status to HIV. Only 5 individuals have been shown to be seropositive and each has practiced risk behavior for HIV infection. In the remainder of the hospital personnel studied, over 332 individuals had experienced some sort of percutaneous or mucosal splash injury with HIV-1 contaminated material and none were found to be seropositive. These data, taken together with other similar nationwide studies, indicate that the risk of infection following percutaneous injury or mucosal splash with HIV-contaminated material is less than 1.0%.

Over the past year, over 100 HIV seropositive, healthy homosexual men have been studied clinically, immunologically, and virologically as part of a protocol for screening candidates for antiretroviral therapy. Of note is the fact that close to 50% of these "healthy" HIV seropositive individuals manifested some degree of immunologic abnormality as evidenced by an inability to mount an in vitro proliferative response to a soluble protein recall antigen such as tetanus toxoid. This was seen even in individuals with normal numbers of T4 lymphocytes confirming our previous studies indicating that the earliest defect in HIV-infected individuals is a selective defect in the antigen-responsive subset of T4 cells. In addition, isolation of virus and detection of viral antigens were more frequent in those patients with lower T4 counts adding credence to the hypothesis that the major reservoir for HIV exists outside the T cell pool and provides additional rationale for studies on the role of the monocyte/macrophage as a reservoir for HIV (see below).

We undertook studies aimed at correlating an immune response profile to HIV with prognosis. We developed isotype-specific enzyme-linked immunosorbent assays that could define antibody responses to the envelope or gag proteins of HIV. We found that among HIV seropositive individuals, the persistence of antibodies to the gag protein was associated with approximately 1/5 the risk of going on to develop AIDS. In contrast, there was no correlation between antibody to envelope protein and subsequent clinical outcome. Among infants of infected mothers, 9 of 18 developed some clinical evidence of immunodeficiency. While all 18 had evidence of IgG antibodies to HIV, presumably due to placental transfer, only the 9 who subsequently developed clinical illness showed an IgM response. Thus, determination of the presence or absence of antibodies to gag as well as the measurement of HIV-specific IgM responses may be of considerable prognostic value.

One of the main areas of interest of the LIR with regard to the immunopathogenesis of HIV infection has been the delineation of the mechanisms of conversion from a latent or low level, chronic infection to a productive infection leading to clinical immunosuppression. We had previously demonstrated that we could chemically (IUdR) induce latently infected lines to express virus. We then demonstrated that antigenic stimulation of mononuclear cell cultures which had been exposed to HIV resulted in a productive infection which did not occur in the absence of stimulation with the antigen. In order to determine if normal physiologically and immunologically relevant stimuli could also play a role in converting a latent to a productive infection, we examined the role of cytokines in inducing virus expression. Because of our interest in cells of the monocyte lineage with regard to their providing a reservoir for HIV, we studied the promonocyte cell line U-937. We established a chronic infection of HIV in U-937 and then developed a panel of cloned, infected lines derived from the original infected U-937 cells. One of these clones (U-1) did not constitutively express HIV. However, upon stimulation with PMA, virus was expressed. We then determined by restriction enzyme analysis that U-1 contained 2 integrated proviral copies in its cellular DNA. We used this U-1 line to determine the effect of cytokines on virus expression as well as the effect of virus infection on the expression of cellular genes. With regard to the former, we demonstrated that a crude supernatant of PHA-stimulated T cells markedly induced virus expression. Recombinant granulocyte/macrophage colony stimulating factor (GM-CSF) alone induced virus expression; however, recombinant IL-1, IL-2, IFN-gamma, and TNF-alpha did not induce virus. Of note was the fact that upon induction of virus expression in U-1 by cytokines or PMA, membrane-bound IL-1 beta expression was upregulated in the absence of upregulation of other cell surface proteins. We also demonstrated upregulation of mRNA for IL-1 beta as well as secreted IL-1 beta. Thus, we have a situation where cellular gene products (cytokines) can upregulate virus expression and virus infection can in turn upregulate expression of cellular gene function (membrane-bound IL-1 beta).

We had previously demonstrated that noninfectious exposure of mononuclear cells to virus resulted in a suppression of proliferative responses to antigens more than to mitogens. It was felt that this was due to the delivery of a tolerogenic or desensitizing signal to the CD4+ T cell. Over the past year, we examined the effects of a noncytopathic infection of CD4+ cells on expression of certain cellular genes critical to immune competence. In these experiments, T3+,T4+ lymphoyctes were infected with HIV. After 2 weeks in culture, a population of infected cells remained which were T3+, T4-, T8-. Using this population, which likely closely approximates the results of in vivo infection with HIV, we demonstrated that the normal mode of transmembrane activation (PMA+PHA) was able to induce the expression IL-2 receptors and IFN-gamma, but was unable to induce expression of IL-2. It is of interest that the expression of IL-2 is downregulated in T cells by HIV in these experiments, and the expression of IL-1 is upregulated by HIV infection in the U-1 clone mentioned above. These observations may provide insight into understanding the mechanisms of pathogenic effects of

HIV infection.

We have extended our studies on the direct activation of human B cells by HIV. Using flow cytometry and cell sorting, highly purified populations of B cells were obtained, and it appears that the HIV-induced proliferation is a totally T cell-independent phenomenon. In situ hybridization studies indicated that the B cells were not infected, and the effect could not be blocked with anti-T4a or human polyvalent HIV. Psoralen-treated virus was able to cause B cell activation at the same level as untreated virus. These findings all point to a membrane triggering of B cell activation in the absence of infection, perhaps mediated by an as yet unidentified cell surface receptor.

Using in situ hybridization techniques with 35-S labeled probes to detect HIV mRNA expression, we have identified the monocyte/macrophage as the major cell type supporting HIV replication in the brain. We have also studied the level of HIV expression by in situ hybridization in the peripheral blood, bone marrow, lung, and gastrointestinal tract. Further analysis of the cell types infected in these tissues are underway.

We have been attempting to understand the virus-specific immunologic effector mechanisms that are generated in the host following infection with HIV. Despite having a panel of excellent reagents, we have been unable to convincingly demonstrate an in vitro antigen-specific proliferative response to viral antigen. Envelope protein (gp120) was capable of inducing polyclonal cellular activation in some individuals, but this was not correlated with seropositivity. Similarly, these proteins have not been found to be significantly immunosuppressive.

Over the past year, we have focused our efforts on the development of a system to measure cytotoxic T cell responses against HIV-infected targets. We have employed EBV-transformed lymphoblastoid B cells infected with recombinant vaccinia virus expressing different portions of the HIV genome. In this way, we could study a wide range of HLA specificities to determine restriction and we could determine precisely the antigens of the virus involved in cytotoxic T cell recognition. Using this system, envelope-specific cytotoxicity was detected in the majority of healthy HIV seropositive and AIDS-related complex patients. The precise major histocompatibility restriction of this system remains to be determined; however, it is clear that syngeneic cells provide a better target than does allogeneic cells.

In the area of treatment, we have focused both on the therapy of opportunistic infections and HIV infection. With regard to the former, we are currently recruiting patients for a placebo-controlled trial of DHPG, a nucleoside analog with potent activity against cytomegalovirus (CMV). It has already been shown to be effective in CMV retinitis, and the current trial is directed towards patients with CMV colitis. In addition, we have just completed a phase II study of trimetrexate, a dihydrofolate reductase inhibitor, which has shown efficacy in the treatment of patients with Pneumocystis carinii pneumonia.

In our studies on the treatment of HIV infection, we are concentrating on early stage patients. We have just completed a placebo-controlled trial of azidothymidine (AZT) in AIDS patients with Kaposi's sarcoma (KS) and >200 T4 cells/mm^3. Preliminary analysis has revealed minor tumor regressions in none of the patients on placebo and 20% of the patients on treatment, a decline in serum HIV antigen levels and a decreased ability to isolate HIV from the CSF of the treated patients. No significant changes were noted in immunologic parameters. Of interest is the fact that 2 patients with over 1000 T4 cells/mm^3 have shown continued tumor regression, now over 6 months on drug. These studies are important in that they are the first to demonstrate that AZT may be of value in the treatment of patients with earlier stages of HIV infection and have served as the basis of studies to be conducted extramurally on the treatment of asymtomatic HIV carriers with AZT.

We are currently conducting an open trial of alpha interferon in AIDS patients with KS who are culture positive for HIV. Fourteen patients have completed the trial, and seven of these have manifested either a complete or partial anti-tumor response. Of note is the fact that all of the patients with an anti-tumor effect had >150 T4 cells/mm^3 at entry while all of the nonresponders had <100 T4 cells/mm^3. In addition, while no changes in HIV antigen levels or isolation were noted in the nonresponders, 4 of 7 of those with a clinical response showed a decline in HIV antigen levels and HIV culture. These data support a role for alpha interferon as an anti-HIV agent in early stages of disease.

The LIR was one of the first laboratories in the world to explore the role of cellular reconstitution of the immune system in AIDS, employing identical twin bone marrow transplantation and syngeneic lymphocyte transfusions. In our earlier studies, this was carried out either alone or in combination with the antiretroviral agent suramin, which later proved to be unsafe and ineffective. In these studies, there was transient improvement in immune function as indicated by increase in T4 count and reconstitution of delayed type cutaneous hypersensitivity. These changes were transient although improvement lasted over a year in 1 patient. In all cases, immune parameters eventually returned to baseline, presumably due to the persistence of HIV and consequent destruction of the partially reconstituted immune system. Our studies over the past year have combined treatment with AZT with bone marrow transplant and syngeneic lymphocyte transfusions between identical twins. The current trial includes AIDS patients, ARC patients, and asymptomatic HIV seropositive patients. Nineteen patients have entered the study, and 11 transplants have been performed since January 1987. It is too early to comment on results.

Finally, we hope to begin phase I vaccine trials this year using recombinant truncated gp160 HIV protein which has been shown to elicit high titers of anti-HIV antibodies in mice, guinea pigs, and nonhuman primates (monkeys and chimps). (Lane, Folks, Koenig, Schnittman, Massari, Poli, Fauci, LIR/NIAID); Masur, Henderson, CC/NIH; Broder, Fox, NCI; Greene, Dukovich, Duke University; Martin, Rabson, LMM/NIAID; Moss, LVD/NIAID; Kottler, Columbia University College of Physicians and Surgeons)

International Studies of the Acquired Immunodeficiency Syndrome

AIDS has become a global pandemic, with cases reported in 113 countries throughout the world. An intensive research effort has been undertaken in several countries in the Caribbean, Africa, India, and South America, to study the unique epidemiologic, virologic, clinical, and immunologic features of AIDS in these areas. In Kinshasa, Zaire, we have identified over 6,000 cases in 1986 with a male to female ratio of 1:1.3. The disease is predominantly heterosexually transmitted with high seroprevalence rates among spouses of AIDS patients, female prostitutes and sexually transmitted diseases (STD) clinic populations. The presence of genital ulcerations, history of oral contraceptives and failure to use condoms are all significantly associated with heterosexual transmission. Additional studies within the U.S. have also confirmed the increasing risk of heterosexual transmission. In studies of perinatal transmission of HIV, advanced clinical disease and immunologic impairment with depressed T4 cells with reversed T4/T8 ratio are adversely associated with enhanced HIV transmission from mother to infants. In Africa, HIV infection in pregnancy is associated with premature birth, low birth weight, and increased mortality (11%) within the first three months of life. Additional serologic and virologic studies have confirmed the presence of a newly identified human retrovirus, referred to as HIV-II, prevalent among patients attending STD clinics. Natural history studies of HIV in Africa have confirmed a 2% annual seroconversion rate among the general population and a clinical progression rate similar to that observed in the U.S. Further studies will continue to examine the natural history of HIV infection in patients of developing countries with emphasis on perinatal transmission, safety and efficacy of immunization programs, and genomic changes of viral isolates from diverse regions. (Quinn, Folks, Francis, Kline, Fauci, LIR/NIAID; Curran, CDC; Piot, Colebunders, Antwerp, Belgium; Holmes, Kreiss, University of Washington)

Clinical, Immunopathogenic, and Therapeutic Studies in the Spectrum of Vasculitis

The LIR is currently studying prospectively one of the largest groups of patients with the vasculitic syndromes in the world. On the basis of clinical, pathophysiologic, immunopathogenic, and therapeutic results obtained over the last 19 years, we have designed a revised categorization scheme for the vasculitides which has now reached worldwide acceptance. On the basis of our observations, lymphomatoid granulomatosis is classified as a subset of malignant lymphoma and not as a primary vasculitis. We have described a new vasculitic syndrome termed the polyangiitis overlap syndrome. We have developed and instituted aggressive chemotherapeutic regimens consisting of chronically administered cyclophosphamide together with corticosteroids. This therapy has dramatically altered the 5-yr survival in patients with systemic vasculitis from 13% in untreated patients to 48% after treatment with corticosteroids to greater than 90% in our patients treated with both cyclophosphamide and corticosteroids. This therapeutic protocol was originally evaluated in our 140 patients with Wegener's granulomatosis but has been extrapolated and successfully used in treating other vasculitides such as polyarteritis nodosa, allergic angiitis and granulomatosis, isolated central nervous system vasculitis, Takayasu's arteritis, the acute vasculitis of Sjögren's syndrome, and the polyangiitis overlap syndrome. Our therapeutic protocols for the vasculitic syndromes are now used worldwide. We have observed certain unexpected longterm side effects of cyclophosphamide and are currently developing new therapeutic protocols for the vasculitic syndromes. In this regard, we have begun to treat patients who cannot tolerate or fail to respond to cyclophosphamide therapy with cyclosporin A. In addition, we have demonstrated the sensitivity of certain phases of the B cell cycle to corticosteroids, cyclophosphamide, and cyclosporin A. These observations might explain the efficacy of these agents in certain diseases characterized by hyperreactivity of B cell function. (Fauci, Leavitt, LIR/NIAID; Parrillo, Shelhamer, CC/NIH; Cupps, Katz, Georgetown University)

Studies in Other Immune-Mediated Diseases

We have extended our studies on the idiopathic hypereosinophilic syndrome (HES) and have recently had the opportunity to investigate a large family cohort of patients with hereditary HES. They will serve as a valuable model for study of this interesting syndrome. We have demonstrated the presence of antineutrophil antibodies in patients with Wegener's granulomatosis and other of the vasculitic syndromes. In some cases, the titer of antineutrophil IgG correlated with disease activity. Finally, we have continued our studies of idiopathic dilated cardiomyopathy and preliminary analysis of data indicate that this disease is responsive to immunosuppressive therapy. (Fauci, Leavitt, Ambrus, LIR/NIAID; Parrillo, CC/NIH; Faroline, Logue, SUNY, Buffalo)

Immunopathogenesis of Chlamydia trachomatis Infection

Chlamydia trachomatis is the most common sexually transmitted bacterial pathogen in the U.S. with an estimated 10 million cases annually. Studies have been carried out to further define the clinical spectrum of chlamydial infection, to develop rapid diagnostic assays, and to examine the pathogenesis of chlamydial infections in experimental animal models. In a study of 704 homosexual men at risk for AIDS, 7.5% had rectal chlamydial infections. Men with chlamydia were more likely to be HIV seropositive (48%) than men without chlamydial infection (25%), to have fewer T helper cells, and to have a mucopurulent exudate. Chlamydial infection rates were markedly increased in those with symptomatic proctitis (12%) and with AIDS (14%). In women, chlamydia cervical infection continues to be high (20%). Coinfection with gonorrhea and human papillomavirus is being examined in relationship to cervical dysplasia and cervical carcinoma by culture and in situ hybridization techniques. The reliability of in situ hybridization utilizing DNA and RNA probes for C. trachomatis has been demonstrated in tissue biopsies from infected animals and patients. Development of a primate model of rectal lymphogranuloma venereum (LGV) infection and respiratory chlamydia-TWAR infection has aided in the study of the mucosal immune response to chlamydial infection. In LGV infection, antigen-reactive T cells are present in the gut mucosa. The administration of cyclosporine inhibited the primary antibody and proliferative response of peripheral blood lymphocytes, but did not prevent the expansion of antigen-specific mucosal and spleen lymphocyte populations. In summary, the above studies demonstrate the diverse clinical spectrum of and provide methods for study of the immunopathogenesis of chlamydial infection. (Quinn, LIR/NIAID; James, Strober, Zeitz, LCI/NIAID; Hook, Horn, Kappus, Rapoza, Taylor, Johns Hopkins Hospital)

The Molecular Biologic Approach to the Immune System

The LIR is pursuing studies on the activation and regulation of the human immune response at the molecular level. In particular, we have exmained the activation of human peripheral blood T cells from a molecular perspective. Through the use of the powerful cloning and screening techniques, we have been able to isolate a large collection of genes which are rapidly induced in human T cells upon mitogenic stimulation. These powerful molecular techniques include the creation of a subtractive cDNA library enriched for induced genes as well as screening with subtractive and differential probes. The rationale behind this approach is the likely identification of new as yet undefined lymphokines and oncogenes as well as an extensive characterization of the intracelluar events following a mitogenic signal. Thus, we have studied the regulation of these genes with different signals and have been able to group them according to their differential behavior. For example, some genes are very much superinduced in the presence of cycloheximide, others are inhibited by it, some are induced rapidly and transiently, some are inhibited in their induction by cyclosporin A, an immunosuppressive drug, and some are responsive to various cell surface directed antibodies and drugs, whereas others are not. Moreover, they can be differentiated with regard to their tissue specificity. Several genes appear to be inducible in normal lung fibroblast cells upon the addition of serum after serum starvation. Lastly, we have discovered that the HTLV-I virus can induce several of these genes upon an infection of an antigen-responsive T helper cell line. These studies are being extended into an analysis of the effect of the HIV virus, as well as the effects of their trans-activating products, tat I and tat III. Over the past year, we have continued our efforts to study the activation of the IL-2 gene in T cells and the regulation of the c-myc gene. Both genes serve as model systems for the study of the T cell-induced genes. As for IL-2, we have established new techniques to detect regulatory DNA binding proteins. In the case of c-myc, we have been able to demonstrate down regulation of a transfected c-myc construct analogous to the downregulation of the endogenous gene upon differentiation of HL60 cells and U937 cells. We are currently delineating the DNA elements required for this control. Lastly we have continued our efforts to clone a B cell growth factor (HMW-BCGF) and its receptor on B cells. In addition to screening our expression library for antigenic expression, we have initiated a screening procedure based on functional activity of the growth factor as well as its recognition by antibodies following in vitro translation of mRNA in a reticulocyte lysate assay. (Siebenlist, Bressler, Brunvand, Zipfel, Schmidt, Ambrus, Fauci, LIR/NIAID; Kelly, IB/NCI; Rapp, FCRC/NCI)

Future Plans and Objectives

Over the past year, the LIR has made a number of significant advances in the study of the regulation of the human immune response in health and disease. Much of our effort was focused on understanding the mechanisms of regulation of the activation, proliferation, and differentiation of human B cells.

We will continue our studies on BCAF derived from the PEER line, which directly activates resting B cells without crosslinking Ig. We will concentrate our efforts on purifying BCAF and identifying the cellular receptor for the factor. In addition, we will pursue the same strategies to clone the genes for the factor as we have with HMW-BCGF and its receptor.

We will continue our studies on HMW-BCGF and its receptor and will pursue the structure-function relationships of the receptor and its triggering and will further define the assemble and expression of the various components of the receptor on normal B cells and will explore its expression and function in diseases characterized by abnormalities of B cell function such as systemic lupus erythematosus, common variable immunodeficiency, and various B cell tumors.

We will continue our studies on human BCDF and will attempt to identify and characterize the cellular receptor for this factor. In addition, we will purify large quantities of BCDF from our transformed BCDF-producing T cell clone and hopefully obtain enough material for sequencing in preparation for cloning the genes for the factor and its receptor.

We will continue our studies on the role of IL-2 and IL-2 receptors in normal human B cell function. We have demonstrated that the p70 protein is expressed on the Tac- SKW6.4 cell line, which responds to high concentrations of IL-2 suggesting that under certain circumstances B cells may be able to express p70 in the absence of Tac. This line of investigation will be pursued over the coming year.

We have recently demonstrated that lymphotoxin (LT) may be an important growth factor for human B cells. We were able to remove BCGF activity from crude T cell supernatants by antibody to recombinant LT. Over the coming year, we will further pursue the extent to which LT is responsible for the induction of B cell growth by T cell supernatants.

We will pursue our studies on the BCGF-BCGF receptor interactions in patients with common variable immunodeficiency. In particular, we will pursue the possibility that patients who have high expression of HMW-BCGF receptors and who do not respond to HMW-BCGF may have a post signal transduction defect.

We will continue and intensify our studies on the epidemiologic, biologic, molecular biologic, immunologic, clinical, and therapeutic studies in AIDS. In particular, we will pursue our studies on the mechanisms of conversion of a latent HIV infection to a productive infection resulting in immunosuppression. We have developed cloned promonocyte lines which are latently infected and have been shown to be induced by cytokines. Over the coming year, we will explore the relationship between induction of viral expression by cytokines as well as the upregulation and downregulation of cellular gene function by integrated proviral sequences. In addition, we will pursue our studies on the mechanisms whereby exposure to HIV or its products delivers a desensitizing or tolerizing signal to uninfected CD4+ lymphocytes. We will also pursue studies on the mechanism of downregulation of gene function by HIV which infects CD4+ T cells in a cytopathic manner. We will also actively pursue our studies on the role of the monocyte as a reservoir of HIV as well as its role in the initiation and propagation of HIV infection.

We will continue our studies on the immune response to HIV infection, particularly in delineating the nature of HIV-restricted cytotoxic T cell responses to HIV-infected target cells. We will further pursue our studies relating antibody responses to certain components of HIV to the clinical prognosis.

We will continue our clinical trials of therapies aimed at opportunistic infections as well as specific therapies against HIV. We will pursue studies of early intervention with immunomodulators and direct antiretroviral agents in HIV-infected patients, including asymptomatic carriers of HIV. We will continue our identical twin bone marrow transplant studies which we have initiated this year and will combine antiretroviral therapy with immunologic reconstitution.

In keeping with our commitment to determining the nature of and effective immune response to HIV, we will be initiating a phase I trial of recombinant gp160 protein as a vaccine candidate. The purpose of the trial will be for the determination of safety as well as immunogenicity.

Our international studies on AIDS will continue both in Africa as well as in the Caribbean and in certain countries in South America.

Our studies on the clinical immunopathogenic and therapeutic aspects of the spectrum of vasculitis will be accelerated over the coming year. We will be initiating a number of new protocols of alternative therapeutic regimens in these diseases in order to improve upon the already excellent results which we have obtained over the past 19 years with cytotoxic drugs and prednisone.

Studies on the clinical, epidemiologic, and immunopathogenic aspects of chlamydial infection will continue in collaboration with the Johns Hopkins University Medical Center.

Honors, Awards, and Scientific Recognition

Over the past year, members of the LIR, particularly in the persons of Drs. Anthony S. Fauci, H. Clifford Lane, and Thomas C. Quinn, have received a number of awarads and honors.

Dr. Fauci continues to serve on a number of committees of scientific and administrative importance. Over the past year, he was made a member of the advisory gorup of the Institut Scientifique Roussel-Uclaf. In addition, he was made Ex Officio Member of the National Kidney and Urologic Diseases Advisory Board. He was made a member of the International Programme Committee of the 8th International Congress of Immunology, which will be held in Budapest, Hungary, in 1987, and he was made a member of the International Advisory Committee for the IVth International Conference on AIDS, which will be held in Stockholm, Sweden, on June 12-16, 1988. Dr. Quinn was made a consultant for the Institute of Medicine, National Academy of Sciences Panel on AIDS.

Dr. Fauci serves as a member of editorial boards of journals concerned with the areas of immunology, allergy, and infectious diseases. He continues his role as Associate Editor in charge of allergy and immunology for the American Journal of Medicine. He maintains his position on the editorial boards of Clinics in Immunology and Allergy, the Annals of Allergy, The Journal of Immunopharmacology, EOS, Clinical and Experimental Rheumatology, La Ricerca in Clinica e in Laboratorio, Clinical Immunology and Immunopathology, Immunologia Clinica e Sperimentale, Physicians' Journal Update, Immunopharmacology, The Journal of Molecular and Cellular Immunology, and Cellular Immunology. In addition, he continues his appointment as an advisory editor for North America for the journal Thymus and for the Journal of Clinical Immunology. He also continues as a member of the editorial board of the Clinical Immunology Newsletter. Over the past year, Dr. Fauci was appointed to the editorial board of the new journal, AIDS Research and Human Retroviruses. He continues to co-edit with Dr. John I. Gallin the book series ADVANCES IN HOST DEFENSE MECHANISMS. In addition, he co-edits the textbooks CURRENT THERAPY IN ALLERGY, IMMUNOLOGY AND RHEUMATOLOGY and CURRENT THERAPY IN INTERNAL MEDICINE. Of particular note is the fact that he is an editor of the internationally renowned textbook of medicine, HARRISON'S PRINCIPLES OF INTERNAL MEDICINE. Finally, Dr. Fauci has contributed a number of invited chapters covering a variety of subjects for most of the major textbooks of medicine as well as subspecialty textbooks in immunology, allergy, and infectious diseases.

As part of the recognition for scientific accomplishments, a clinical investigator may be asked to visit outside institutions and serve for periods from two to five days as a visiting professor within a given institution. In this regard, Dr. Fauci has served as visiting professor at major medical centers throughout the country over the years. Over the past year, he was The Seventh Annual Visiting Professor of the Division of Geographic Medicine of Case Western Reserve University Medical Center, Cleveland, OH. In addition, he was The First Annual E. Ross Kyger, Jr., M.D. Visiting Professor in the Department of Medicine at the University of Pennsylvania Medical Center in Philadelphia. And finally, he was The Woodward Visiting Professor of the Department of Medicine at Memorial Sloan-Kettering Center in New York. Dr. Lane was visiting professor at the University of Michigan.

Dr. Fauci was asked to deliver several major or named lectureships during the year. Dr. Fauci was an invited plenary speaker at the First International Union of Immunological Societies Conference on Clinical Immunology, the Charter Meeting of the Clinical Immunology Society, the 4th Annual Military Health Professionals Symposium, Colloque des "Cent Gardes" on Retroviruses of Human AIDS and Related Animal Retroviruses, the American Medical

Association National Conference on AIDS and Public Policy: A Community Response, the American Pediatric Society Presidential State of the Art Symposium on AIDS: Special Challenges for Infants an Children, and the American Federation for Clinical Research Annual Meeting, Frontiers in Clinical Science Symposium. Dr. Fauci delivered the prestigious Werner Braun Memorial Lectureship of the Waksman Institute of Microbiology at Rutgers University and the Woodward Lectureship of the Sloan-Kettering Institute. He also delivered the 7th Annual Lecture in Geographic Medicine at Case Western Reserve University, the 1987 Annual Lectureship of the American Society of Microbiology, and the Annual Alpha Omega Alpha Lecture of the Mount Sinai School of Medicine. In addition, he was a symposium speaker at the 6th International Congress of Immunology, the Twenty-Sixth Midwinter Conference of Immunologists, and the Fifth International Lymphokine Workshop. Dr. Fauci's invited plenary lectures include the Annual Meeting of the American Academy of Allergy and Immunology, the Annual Meeting of the Virginia Chapter of the American College of Physicians, Montedison Progretto Cultura in Milan, and the Italy, and Instituto Superiore di Sanita in Rome. He was an invited plenary lecturer at the III Internatonal Conference on AIDS. Dr. Fauci was an invited symposium speaker and chaired a minisymposium at the Annual Meeting of the American Association of Immunologists.

Dr. Lane delivered invited lectures at the Annual Meeting of the American Academy of Dermatology, and the Annual Meeting of the American Academy of Allergy and Immunology. He was a workshop chairman at the annual meeting of the Clinical Immunology Society and the III International Conference on AIDS. Dr. Quinn delivered invited lectures at Stanford University, Duke University, and the University of New Mexico. He also delivered invited lecutres at the Surgeon General's Workshop on Pediatric AIDS, the Biology and Immunopathogenesis of Human Viruses meeting, the Cold Spring Harbor Symposium on Vaccine Development, the FDA Workshop on AIDS Vaccine, the Annual Meeting of the American Society of Microbiology, the Annual Meeting of the American Practitioners of Infectious Control, and the Annual Meeting of the National Council for International Health. In addition, he delivered the AOA Epidemiology Research Award and Lectureship and the Annual Distinguished Physician's Memorial Lectureship. Dr. Thomas Folks was an invited lecturer at the Annual Meeting of the American Society for Microbiology. Dr. Randi Leavitt delivered Pulmonary Grand Rounds at the Bronx Municipal Hospital, and she delivered an invited lecture at the workshop on Immune Mechanisms in the Diseases of Head and Neck.

Finally, over the past year, Dr. Fauci was honored by being elected into the Institute of Medicine of the National Academy of Sciences. In addition, he received a Doctor of Science, Honoris Causa, from the College of the Holy Cross in Worcester, MA. Dr. Lane was awarded the Meritorious Service Medal of the United States Public Health Service and was granted a Fellowship in the Infectious Diseases Society of America.

Administrative, Organization, and Other Changes

The Laboratory of Immunoregulation (LIR) was established in late 1980 and is now seven years old. The theme of the LIR is the study of the mechanism of activation and immunoregulation of the human immune response in normal individuals and in a variety of diseaes characterized by abnormalities of immune function. In addition to basic research, ranging from cellular physiology to molecuar biology, the LIR continues to conduct a major portion of the clinical studies that are carried out in the NIAID Intramural Research Program within the NIH Clinical Center. Over the past year, Dr. H. Clifford Lane has continued to assume major responsibility for conducting the clinical studies on AIDS in the LIR. He continues his role as a Deputy Clinical Director of NIAID as he coordinates this wide range of clinical studies. The clinical staff under Dr. Lane was expanded to include more nurses and support personnel. Dr. Joseph Kovacs, who will assist Dr. Lane in clinical studies on AIDS patients, joined the group. Dr. Robert Walker, who was a full-time clinic physician in Dr. Lane's group, has left for a fellowship in pulmonary medicine at the University of Pennsylvania; he was replaced by Dr. Lawrence Deyton. Dr. Fauci will continue his role as coordinator of AIDS research for the entire NIH. Dr. Randi Y. Leavitt continues her responsibilities in assisting Dr. Fauci to conduct clinical research in the LIR. In July 1987, Dr. Gary Hoffman joined the staff to add extra clinical research expertise for the inflammatory diseases research protocols under Dr. Fauci. Dr. Harris Goldstein has left the LIR for a staff position at Albert Einstein Medical Center. Drs. K. Randall Young and Steven Schnittman have entered their second year of Medical Staff Fellowship and Drs. Scott Koenig and Peter Bressler have entered their third year. Dr. Ferdinand Massari has joined the LIR as a Medical Staff Fellow, and Dr. Guido Poli has joined the laboratory as a Visiting Fellow. Drs. Toshimasa Nakagawa and Naoko Nakagawa have left the LIR. Dr. Alan Taylor has joined Dr. John H. Kehrl as a Staff Fellow. Ms. Lori Abrams is now working with Dr. Lane as an AIDS Protocol Assistant.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00210-07 LIR

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunoregulation of Human Lymphocyte Function in Normal and Disease States

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.S. Fauci	Chief	LIR, NIAID
Others:	J.H. Kehrl	Senior Investigator	LIR, NIAID
	J.L. Ambrus, Jr.	Senior Investigator	LIR, NIAID
	H. Goldstein	Senior Staff Fellow	LIR, NIAID
	U. Siebenlist	Senior Staff Fellow	LIR, NIAID
	K.R. Young	Medical Staff Fellow	LIR, NIAID
	P. Bressler	Medical Staff Fellow	LIR, NIAID

COOPERATING UNITS *(if any)*

Washington Univ. (E.J. Brown, M. Peters); Duke Univ. (W.C. Greene, M. Dukovich); American Red Cross (A. Tenner); Harvard Univ. (R. Lewis, K.F. Austen, M. Fineberg); NCI (S. Rudikoff, A. Roberts, M. Sporn, Le thi Bich-Thuy); Madrid, Spain (M. Alvarez-Mon); Univ. Chicago (M. Gurney);

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7	4	3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have studied the mechanisms of activation, proliferation, and differentiation of human lymphocytes, particularly B cells, at the cellular and molecular levels. We have identified and purified a high molecular weight B cell growth factor (HMW-BCGF) and its cellular receptor (BA5) which is a 90 Kd heterodimer with at least 1 associated protein and which is assembled from smaller precursor proteins. We have demonstrated that HMW-BCGF acts selectively on certain subsets of B cells. B cell proliferation can also be modulated by the Ba and Bb fragments of the complement component factor B, and Clq was also shown to induce IgM secretion by normal B cells. We have purified a 30 Kd B cell differentiation factor (BCDF) from a T4+ clone. We are at various stages of cloning the genes for our HMW-BCGF, its receptor, and the BCDF. We demonstrated that LTC_4 and PGE_2 inhibited Ig secretion establishing a link in our system between the inflammatory and immune responses. We have demonstrated that transforming growth factor (TGF)-ß is a potent inhibitor of B cell activation. We have extended our studies delineating the BCGF activity of tumor necrosis factor (TNF)-α by binding and cross-linking studies of factor to receptor and have demonstrated that there is a marked upregulation of TNF-α receptors following B cell activation. Lymphotoxin (LT), a structurally and functionally related protein to TNF, was also found to have BCGF activity, and Northern blots demonstrated the rapid induction of messenger RNA for LT in mitogen-activated T cells. Using chemical crosslinking with ^{125}I-IL-2, we have identified a second IL-2 binding protein (p70) which is distinct from the Tac protein. The presence of the p70 protein on resting T cells, natural killer (NK) cells, and SKW 6.4 B cell lines suggests that IL-2 binding to this receptor accounts for the previous observations that these Tac negative cells can respond to IL-2. Finally we have demonstrated 2 proteins (p50 and p90) which likely constitute the IFN-γ receptor on B cells.

Others:	A. Taylor	Staff Fellow	LIR, NIAID
	T. Nakagawa	Visiting Fellow	LIR, NIAID
	N. Nakagawa	Visiting Fellow	LIR, NIAID

Cooperating units: Scripps Clinic (M. McChesney); Univ. Illionois (A. Miller, Univ.); Rockefeller (P. Sehgal); Georgetown (P. Katz).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00212-07 LIR

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of the Immunopathogenic Features of Immune-Mediated Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.S. Fauci	Chief	LIR, NIAID
Others:	J.L. Ambrus, Jr.	Senior Investigator	LIR, NIAID
	R.Y. Leavitt	Senior Investigator	LIR, NIAID
	K.R. Young	Medical Staff Fellow	LIR, NIAID
	T. Nakagawa	Visiting Fellow	LIR, NIAID
	N. Nakagawa	Visiting Fellow	LIR, NIAID

COOPERATING UNITS *(if any)*

CC, NIH, (J.E. Parrillo); Mayo Clinic, Rochester, MN, (G. Gleich); Univ. Minnesota, (F. Uckun); Duke Univ., (R. Buckley); Georgetown Univ. (P. Katz, T. Cupps); SUNY, Buffalo, (D. Farolino, G. Logue).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The immunopathogenic mechanisms of a number of immune mediated diseases and/or diseases characterized by abnormalities of immune function were investigated. B cells from patients with common variable immunodeficiency (CVI) were studied and shown to respond normally to low molecular weight B cell growth factor (LMW-BCGF), but abnormally or not at all to high molecular weight (HMW)-BCGF despite the ability to express receptors for HMW-BCGF. This abnormality is compatible with a post receptor signal transduction defect. Indeed, our data suggest that overproduction of an arachidonic acid metabolite such as PGE or one of the leukotrienes may be playing an inhibitory role in the normal signal transduction occurring with HMW-BCGF. Studies in systemic lupus erythematosus (SLE) indicate that T cells of patients who have disease flares with production of IgG antibody produce a factor which enhances IgG production and inhibits IgM production in vitro. We have extended our studies on the hypereosinophilic syndrome (HES) and have recently had the opportunity to investigate a large family cohort of patients with hereditary HES. They will serve as a valuable model for study of this interesting syndrome. We have demonstrated the presence of antineutrophil antibodies in patients with Wegener's granulomatosis, lymphomatoid granulomatosis, polyarteritis nodosa, and SLE. In some cases, the titer of antineutrophil IgG correlates with disease activity. Finally, we have continued our studies of idiopathic dilated cardiomyopathy and preliminary analysis of data indicate that this disease is responsive to immunosuppressive therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00213-07 LIR

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical, Immunopathogenic, and Therapeutic Studies in the Spectrum of Vasculitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.S. Fauci	Chief	LIR, NIAID
Others:	R.Y. Leavitt	Senior Investigator	LIR, NIAID

COOPERATING UNITS *(if any)*

Critical Care Medicine Department, Clinical Center, NIH (J.E. Parrillo, J. Shelhamer); Georgetown University (T.R. Cupps, P. Katz)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The LIR is currently studying prospectively one of the largest groups of patients with the vasculitic syndromes in the world. On the basis of clinical, pathophysiologic, immunopathogenic, and therapeutic results obtained over the last 19 years, we have designed a revised categorization scheme for the vasculitides which has now reached worldwide acceptance. On the basis of our observations, lymphomatoid granulomatosis is classified as a subset of malignant lymphoma and not as a primary vasculitis. We have described a new vasculitic syndrome termed the polyangiitis overlap syndrome. We have developed and instituted aggressive chemotherapeutic regimens consisting of chronically administered cyclophosphamide together with corticosteroids. This therapy has dramatically altered the 5-yr survival in patients with systemic vasculitis from 13% in untreated patients to 48% after treatment with corticosteroids to greater than 90% in our patients treated with both cyclophosphamide and corticosteroids. This therapeutic protocol was originally evaluated in our 140 patients with Wegener's granulomatosis but has been extrapolated and successfully used in treating other vasculitides such as polyarteritis nodosa, allergic angiitis and granulomatosis, isolated central nervous system vasculitis, Takayasu's arteritis, the acute vasculitis of Sjögren's syndrome, and the polyangiitis overlap syndrome. Our therapeutic protocols for the vasculitic syndromes are now used worldwide. We have observed certain unexpected longterm side effects of cyclophosphamide and are currently developing new therapeutic protocols for the vasculitic syndromes. In this regard, we have begun to treat patients who cannot tolerate or fail to respond to cyclophosphamide therapy with cyclosporin A. In addition, we have demonstrated the sensitivity of certain phases of the B cell cycle to corticosteroids, cyclophosphamide, and cyclosporin A. These observations might explain the efficacy of these agents in certain diseases characterized by hyperreactivity of B cell function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00390-04 LIR

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of the Acquired Immunodeficiency Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H.C. Lane	Senior Investigator	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	T.M. Folks	Expert	LIR, NIAID
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COOPERATING UNITS *(if any)*

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LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
11	6	5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An intensive effort was directed at studying epidemiologic, virologic, immunologic, and clinical aspects of the acquired immunodeficiency syndrome (AIDS). An ongoing study of over 500 hospital employees has continued to shown a low risk of hospital acquired infection with HIV despite percutaneous or mucosal exposure. Quantitative analysis of isotype specific antibody responses to gag and env gene products of HIV revealed a better prognosis for adult individuals with anti-gag and increased problems for infants of HIV-1 positive mothers who develop IgM anti-env or anti-gag responses. Over 50 per cent of asymptomatic HIV positive individuals were found to have some abnormality of immune function. Over 100 different clinical isolates of HIV have been obtained with no correlation seen between the presence of virus in spinal fluid and clinical neurologic disease. A variety of promonocyte lines were found capable of harboring latent HIV-1 which could be induced with various cytokines. Induction of HIV-1 was associated with induction of IL-1. Peripheral blood CD4 cells infected with HIV-1 were found to be defective in their ability to transcribe IL-2 following stimulation with phorbol ester, but not following stimulation with calcium ionophore. The HIV-induced activation of B cells in vitro was found to be independent of viral infection. Studies utilizing in situ hybridization revealed the presence of HIV RNA in brain, lung, and colonic tissue, usually in association with macrophages. The fine specificity of HIV-specific cytotoxic T cells was examined utilizing lymphoblastoid cell lines infected with recombinant vaccinia viruses expressing different parts of the HIV genome. The HIV specific immune responses of chimps following vaccination with gp160 was studied at the level of T cell blast transformation and neutralizing antibody production. A series of clinical studies were conducted in an attempt to improve the clinical status of patients with AIDS. AZT and alpha interferon were found to have clinical and anti-viral effects in patients with early immunodeficiency. Trials of muramyl tripeptide, bone marrow transplantation with AZT and AZT with alpha interferon are in progress.

Others:	M. Megill	Nurse Practitioner	LIR, NIAID
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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00431-03 LIR

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Molecular Biologic Approach to the Immune System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	U.K. Siebenlist	Senior Staff Fellow	LIR, NIAID
Others:	P. Bressler	Medical Staff Fellow	LIR, NIAID
	M.W. Brunvand	National Research Service Award Fellow	LIR, NIAID
	P.F. Zipfel	Guest Researcher	LIR, NIAID
	A.G. Schmidt	Guest Researcher	LIR, NIAID
	J.L. Ambrus, Jr.	Senior Investigator	LIR, NIAID
	A.S. Fauci	Chief	LIR, NIAID

COOPERATING UNITS *(if any)*

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LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	5	1

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☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have studied the activation of human peripheral blood T cells from a molecular perspective. Previously we have constructed a subtractive cDNA library and used subtractive and differential screening to isolate genes which are induced early on in T cells stimulated by mitogens. Such genes are likely to include new lymphokines and receptors as well as genes which control proliferation and thus may have oncogenic potential. So far, we have isolated more than 53 individual genes induced by mitogens in T cells and these include such genes as the c-myc oncogene and the interleukin-2 (IL-2) receptor gene. Also, we have studied the regulation of these genes. They can be grouped by their kinetics of induction, by their behavior in the presence of cycloheximide, by their sensitivity to cyclosporin A (an immunosuppressive drug) and by their inducibility with various distinct mitogens or activators (T cell receptor antibodies, IL-2, calcium ionophores, 13-phorbol-12-myristate acetate alone, phytohemagglutinin alone). Furthermore, some of these genes appear to be T cell specific, whereas others are inducible also in human fibroblsts. Lastly, we have discovered that some of these genes are activated by the human T lymphotropic virus I. We are now studying the effect of the tat I transactivating protein of this virus as well as the effect of the human immunodeficiency virus virus and its tat III protein on the activation mechanism in T cells. In addition to these studies, we have continued our efforts to understand the molecular mechanisms leading to the activation of the IL-2 gene in T cells, as well as the events leading to downregulation of c-myc during differentiation of HL60 and U937 cells. We have successfully transfected these latter cells with a c-myc promoter construct which can be regulated. Therefore, we have a model system in hand to understand the molecular elements required for this downregulation. Lastly, we have initiated new approaches to clone the high molecular weight B cell growth factor discovered in the LIR and characterized in other project reports.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00361-05 LIR

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

International Studies of the Acquired Immunodeficiency Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.C. Quinn	Senior Investigator	LIR, NIAID
Other:	A.S. Fauci	Chief	LIR, NIAID
	T.M. Folks	Expert	LIR, NIAID
	H.L. Francis	Expert	LIR, NIAID
	R. Kline	Microbiologist	LIR, NIAID

COOPERATING UNITS *(if any)*

CDC, (J. Curran); Institute Tropical Medicine, Antwerp, Belgium, (P. Piot, R. Colebunders); Univ. of Washington, (K. Holmes, J. Kreiss).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	1

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☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The acquired immunodeficiency syndrome (AIDS) has become a global pandemic, with cases reported in 113 countries throughout the world. An intensive research effort has been undertaken in several countries in the Caribbean, Africa, India, and South America to study the unique epidemiologic, virologic, clinical, and immunologic features of AIDS in these areas. In Kinshasa, Zaire, we have identified over 6,000 cases in 1986 with a male to female ratio of 1:1.3. The disease is predominantly heterosexually transmitted with high seroprevalence rates among spouses of AIDS patients, female prostitutes, and sexually transmitted diseases (STD) clinic populations. The presence of genital ulcerations, history of oral contraceptives and failure to use condoms are all significantly associated with heterosexual transmission. Additional studies within the U.S. have also confirmed the increasing risk of heterosexual transmission. In studies of perinatal transmission of human immunodeficiency virus (HIV), advanced clinical disease and immunologic impairment with depressed T4 cells and reversed T4/T8 ratio are adversely associated with enhanced HIV transmission from mother to infants. In Africa, HIV infection in pregnancy is associated with premature birth, low birth weight, and increased mortality (11%) within the first 3 months of life. Additional serologic and virologic studies have confirmed the presence of a newly identified human retrovirus, referred to as HIV-II, prevalent among patients attending STD clinics. Natural history studies of HIV in Africa have confirmed a 2% annual seroconversion rate among general population and a clinical progression rate similar to that observed in the U.S. Further studies will continue to examine the natural history of HIV infection in patients of developing countries with emphasis on perinatal transmission, safety and efficacy of immunization programs, and genomic changes of viral isolates from diverse regions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00358-05 LIR

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunopathogenesis of Chlamydia trachomatis infection.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.C. Quinn	Senior Investigator	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID

COOPERATING UNITS *(if any)*

LCI, NIAID, (S. James, W. Strober, M. Zeitz); Johns Hopkins University, (E. Hook, J. Horn, E. Kappus, P. Rapoza, and H. Taylor).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS: 1	PROFESSIONAL: 1	OTHER: 0

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☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Chlamydia trachomatis is the most common sexually transmitted bacterial pathogen in the U.S. with an estimated 10 million cases annually. Studies have been carried out to further define the clinical spectrum of chlamydial infection, to develop rapid diagnostic assays, and to examine the pathogenesis of chlamydial infections in experimental animal models. In a study of 704 homosexual men at risk for AIDS, 7.5% had rectal chlamydial infections. Men with chlamydia were more likely to be HIV seropositive (48%) than men without chlamydial infection (25%), to have fewer T helper cells, and to have a mucopurulent exudate. Chlamydial infection rates were markedly increased in those with symptomatic proctitis (12%) and with AIDS (14%). In women, chlamydia cervical infection continues to be high (20%). Coinfection with gonorrhea and human papillomavirus is being examined in relationship to cervical dysplasia and cervical carcinoma by culture and in situ hybridization techniques. The reliability of in situ hybridization utilizing DNA and RNA probes for C. trachomatis has been demonstrated in tissue biopsies from infected animals and patients. Development of a primate model of rectal C. trachomatis lymphogranuloma venereum (LGV) infection and respiratory chlamydia-TWAR infection has aided in the study of the mucosal immune response to chlamydial infection. In LGV infection, antigen-reactive T cells are present in the gut mucosa. The administration of cyclosporine inhibited the primary antibody and proliferative response of peripheral blood lymphocytes, but did not prevent the expansion of antigen-specific mucosal and spleen lymphocyte populations. In summary, the above studies demonstrate the diverse clinical spectrum of C. trachomatis and provide methods for study of the immunopathogenesis of chlamydial infection.

LID

LABORATORY OF INFECTIOUS DISEASES
1987 ANNUAL REPORT

Table of Contents

Z01 AI Project Number		Page
Summary		11-01
Hepatitis Viruses Section		
00308-06	*In Vitro* and *In Vivo* Studies of Hepatitis A Virus -- Karron	11-35
00309-06	Structural and Antigenic Analysis of Hepatitis A Virus -- Feinstone	11-36
00311-06	Search for New Hepatitis Agents -- Ticehurst	11-37
00312-06	Clinical and Experimental Studies of Hepatitis B Vaccines -- Purcell	11-38
00314-06	Woodchuck Virus -- Miller	11-39
00316-06	The Delta Agent -- Purcell	11-40
00317-06	Biology of Non-A, Non-B Hepatitis Agents -- Feinstone	11-41
00319-05	New Approaches to *In Vitro* Propagation of Non-Cultivatable Agents -- Purcell (Terminated)	11-42
00321-06	Development, Characterization, and Use of Cloned Hepatitis A Virus HAV cDNA -- Ticehurst	11-43
00370-05	Studies of Acquired Immune Deficiency Syndrome (AIDS) -- Purcell	11-44
00404-04	Second and Third-Generation Hepatitis B Vaccines -- Purcell	11-45
00405-04	Inactivation of Hepatitis Viruses in Pooled Plasma Derivatives -- Purcell	11-46
00406-04	Molecular Structure of HAV: Genomic Sequence and Organization -- Cohen	11-47
00440-03	Construction of Full Length Hepatitis A Virus cDNA for Transfection -- Cohen	11-48
00473-02	Replication of Hepatitis A Virus *In Vivo* -- Cohen	11-49

Respiratory Viruses Section

00323-06 Structure and Expression of Parainfluenza Type 3 Virus Genes -- Collins 11-50

00324-06 Laboratory Studies of Myxoviruses -- Snyder 11-51

00325-06 Study of Respiratory Viruses in Primates -- Murphy 11-52

00326-06 Study of Respiratory Viruses in Volunteers -- Snyder 11-53

00327-06 Characterization of Parainfluenza Type 3 Virus Surface Glycoproteins -- Coelingh 11-54

00344-06 Immunologic Factors in Respiratory Syncytial Virus Disease -- Murphy 11-55

00345-06 Immunity to RS Virus -- Murphy 11-56

00368-05 Structural Analysis of Respiratory Syncytial Virus Genome -- Collins 11-57

00372-05 RSV Proteins: Roles in Host Immunity and Immunoprophylaxis -- Collins 11-58

00457-03 Human Immune Response to Respiratory Syncytial Virus Virus Infection or Vaccination -- Murphy 11-59

00474-02 Molecular Mechanisms Underlying Adenovirus Pathogenesis -- Ginsberg 11-60

00497-01 Characterization of the Fusion Glycoprotein of Respiratory Syncytial Virus -- Coelingh 11-61

00498-01 Synthesis, Processing and Functions of the Proteins of Human RSV -- Collins 11-62

Molecular Viral Biology Section

00332-05 Engineering the Genome of Influenza Virus -- Lai (Terminated) 11-63

00365-04 Functional Analysis of Signal Sequences in Influenza Hemagglutinin -- Lai (Terminated) 11-64

00366-05 Molecular Biology of Dengue Viruses -- Zhao and Lai 11-65

00407-03 Complementation of Influenza Mutants by Cloned Viral Genes -- Ryan (Terminated) 11-66

00408-03 Attempts at Allele Replacement of NA Gene of Influenza A Virus -- Markoff (Terminated) 11-67

00458-02	Genetic Variations Among Dengue Viruses -- Zhang (Inactive)	11-68
00459-03	Expression of Dengue Virus Proteins Using Baculovirus as a Vector -- Zhang	11-69
00475-02	Nucleotide Sequence of Genes for Dengue Virus Type 4 Non-Structural Proteins -- Zhao	11-70
00476-02	Engineering the Genome of Dengue Virus -- Lai	11-71
00477-02	Expression of Dengue Viral Genes and Vaccinia Recombinant Virus -- Zhao and Lai	11-72
00499-01	Expression of Selected Dengue E Protein Sequences by Recombinant Vaccinia Virus -- Bray	11-73
00500-01	Immunogenicity of Dengue Type 4 Virus Nonstructural Protein NS1 -- Falgout	11-74
00501-01	Study of the Processing of Dengue Viral Glycoproteins -- Markoff	11-75
00502-01	Antigenic Analysis of the Dengue Envelope Glycoprotein Using Synthetic Peptides -- Markoff	11-76
00503-01	Expression of Dengue Virus Antigens Using a Synthetic Signal Peptide -- Markoff	11-77

Epidemiology Section

00333-06	A Longitudinal Study of Viral Gastroenteritis in Infants and Young Children -- Kapikian	11-78
00334-05	Development of Monoclonal Antibodies to Rotavirus Proteins -- Midthun (Terminated)	11-79
00335-06	Rotavirus Reassortants: Genetics and Use in Rotavirus Vaccines -- Midthun	11-80
00338-06	Molecular Cloning of Rotavirus Genes -- Flores	11-81
00339-06	Isolation and Serotypic Characterization of Human and Animal Rotaviruses -- Hoshino	11-82
00340-06	Experimental Studies in Animals with Various Rotaviruses and Their Reassortants -- Hoshino	11-83
00341-06	Evaluation of Experimental Rhesus Rotavirus Vaccine in Infants and Children -- Kapikian	11-84
00342-06	Studies of Gastroenteritis Viruses by Electron Microscopy -- Kapikian	11-85

00343-06	The Role of Norwalk-Like 27nm Virus Particles in Viral Gastroenteritis -- Midthun	11-86
00346-06	Genetic Characterization of Rotavirus by Hybridization Techniques -- Flores	11-87
00446-03	Testing of Rotavirus Vaccine Candidates in Venezuela -- Flores	11-88
00450-03	Sequence of the Fourth Rotavirus Gene and Its Role in Virulence -- Gorziglia	11-89
00451-03	Immunological Characterization of Rotavirus Antigens -- Hoshino	11-90
00478-02	Development of Adenovirus Expression Vectors -- Flores	11-91
00479-01	Development of Monoclonal Antibodies to Proteins of Rotavirus -- Hoshino (Merged with 00451-03 and Terminated)	11-92
00504-01	Sequence of the Sixth Rotavirus Gene -- Gorziglia	11-93
00505-01	Neutralization Epitopes on Rotavirus Outer Capsid Proteins -- Gorziglia	11-94
00506-01	Sequencing of Rotavirus Genes -- Green	11-95
00507-01	Molecular Characterization of Two New Human Rotavirus Serotypes -- Green	11-96
00508-01	Naturally Occurring Rotavirus Reinfection -- Midthun	11-97
00509-01	Crossreactive Neutralizing Epitopes on Rotavirus Outer Capsid Protein VP7 -- Nishikawa	11-98
00510-01	Rotavirus VP7 Neutralization Epitopes of Serotype 3 Strains -- Nishikawa	11-99
00511-01	Rotavirus Cross-Reactive and Serotype-Specific Neutralization Epitopes -- Taniguchi	11-100

SUMMARY STATEMENT
Annual Report
Laboratory of Infectious Diseases
National Institute of Allergy and Infectious Diseases
October 1, 1986 to September 30, 1987

Introduction

The research goals of the LID remain: (a) delineation of the etiology, pathogenesis and epidemiology of medically important viral diseases and (b) development of means for their control. Although the mission of LID has not changed, our research efforts have broadened as advances in molecular biology have provided new technological opportunities to explore medically important viruses at a more basic level. The newer technology has allowed us to define structure, organization and function of a number of viral genomes and to express individual viral proteins which were then evaluated for their role in immunity and usefulness in immunoprophylaxis. Various noncoding regions of the viral genome or individual viral proteins are now being evaluated at the molecular level for their role in pathogenesis and virulence. Rather remarkable success has been achieved in each of these areas of research during the past year, but our overriding concern remains the need to relate these new insights to the understanding and prevention or treatment of viral disease. In this regard, there is cause for optimism that a number of important viral pathogens, such as hepatitis A virus (HAV), respiratory syncytial virus (RSV), parainfluenza type 3 virus (PIV3), dengue virus and rotavirus, will soon be brought under control by immunization with an attenuated viral mutant or a viral protein expressed in a recombinant vector.

An additional major program was recently initiated within LID. This research effort will be devoted to a study of the simian and feline immunodeficiency retroviruses (SIV and FIV, respectively) in their natural host. An attempt will be made to identify: (a) protective antigens among the viral proteins of these retroviruses and (b) immune correlates of resistance to infection. Specifically, we will determine whether passively transferred specific neutralizing and/or ADCC antibodies can provide effective resistance to infection by these viruses. If the answer is yes, we will then quantitate these antibodies to determine the amount required for effective resistance. This information should be useful in evaluating various experimental approaches to immunization against these immunodeficiency retroviruses which represent surrogates for the human AIDS virus, HIV. These studies will be performed within an enlarged Hepatitis Viruses Section because Dr. Purcell and his associates have had a long experience and many notable successes in the investigation of chronic viral infections in primates.

Hepatitis Viruses

Hepatitis A Virus (HAV): Attenuated Tissue Culture Adapted HAV Mutant. The hepatitis A virus (HAV), the etiologic agent of epidemic hepatitis, is a picornavirus that was isolated in tissue culture for the first time relatively recently. Previously in another laboratory, passage of human clinical materials in marmoset monkeys was necessary before HAV could be isolated in tissue culture. Recently, we isolated a strain of HAV directly in African green monkey kidney (AGMK) cells, a cell type licensed for use in production of human viral vaccines. Cell culture adapted HAV was thrice cloned at two different passage levels, 21 and 32, by terminal dilution and radioimmunoassay was used to identify positive cultures. This strategy was required because the virus is largely cell associated and generally is not cytopathic in cell culture. The two cloned stocks of tissue culture-adapted HAV were characterized in chimpanzees and marmosets and were observed to be attenuated compared to wild-type virus. Significantly, the attenuated virus did not revert to virulence during serial passage through chimpanzees. Immunization of chimpanzees with the passage 32 tissue culture adapted HAV induced effective resistance to challenge with wild-type, virulent HAV (Karron, Feinstone, Purcell).

HAV Antigens. Antibodies to hepatitis A viral structural proteins were induced with SDS disrupted purified HAV or synthetic peptides. These antibodies were then used to analyze HAV proteins by Western blot, virus neutralization, and radioimmunoassay. It was observed that cleavage of precursor protein VP0 into capsid proteins VP2 and VP4 did occur, but the fate of VP4 in the mature virion is still unclear. Neutralization epitopes were sought by analyzing antibodies to synthetic oligopeptides of HAV VP1 for neutralizing activity. Only one of these antibodies neutralized HAV at a very low level. Two epitope libraries were constructed in lambda gt 11 but attempts to detect clones which synthesized a peptide recognized by neutralizing monoclonal antibody were unsuccessful. Thus, it appears that most HAV neutralization epitopes are probably discontinuous rather than linear (Feinstone, Karron).

Sequence Analysis of HAV. The complete nucleotide sequence of the wild-type HAV, strain HM-175 was determined and compared to the complete nucleotide sequence of the attenuated mutant vaccine candidate, derived from wild-type virus by 32 passages in AGMK cells. Comparison of the nucleotide sequence of cDNA from wild-type virus with that of its attenuated mutant revealed 24 nucleotide changes, some or all of which are responsible for attenuation. Five base deletions and two point mutations occurred in the 5' noncoding region. As a result, the viral RNA in the 5' noncoding region of attenuated HAV differs from that of its wild type parent in predicted secondary structure; one of the point mutations in this region (base 124) is responsible for most of the difference in predicted secondary structure. Similar changes in predicted secondary structure were identified previously in the 5' noncoding viral RNA of attenuated vaccine strains of poliovirus type 1 and type 3. Sixteen nucleotide changes also occurred in the coding region of the attenuated HAV, resulting in 12 amino acid changes. One nucleotide change in the capsid region resulted in the substitution of an amino acid of different charge. Ten amino acid changes occurred in the nonstructural proteins and differences in amino acid charge were seen in proteins 2B, 2C, 3B, and 3D. One nucleotide change occurred in the 3' noncoding region and one of three cDNA clones of attenuated HAV had an additional base at the 3' end of the genome (Cohen, Ticehurst, Feinstone, Purcell).

cDNA Hybridization Probes. Hybridization probes of HAV cDNA were used successfully for detection of HAV RNA in tissue culture, liver, serum, and fecal specimens. Hybridization with cDNA was more sensitive for detection of HAV than was a radioimmunoassay (RIA) for viral proteins. Analysis of a large number of hepatitis A samples from a 1974 outbreak in San Diego, CA and from sporadic cases in Greece demonstrated that HAV RNA was detectable for a longer period during infection than were viral antigens. Hybridization is also being used to measure virus replication in animals which were inoculated with attenuated HAV mutants. Fecal extracts from a small number of patients with epidemic non-A/non-B hepatitis failed to hybridize with HAV cDNA probes representing most of the viral genome (Ticehurst, Feinstone, Purcell).

Genome Linked Protein (VPg). During an analysis of amino acid homologies among picornaviruses, a sequence was identified in the P3 region of the HAV polyprotein that had the major features of the genome linked protein (VPg) present in other picornaviruses. Antibodies were raised in rabbits to a synthetic peptide that represented the 10 C-terminal amino acids of the putative VPg sequence (anti-cVPg). Immunoprecipitation with these antibodies demonstrated that purified HAV particles and HAV-infected cells contain a VPg that is linked to viral RNA (Weitz, Ticehurst).

Construction of Infectious HAV cDNA. During the past year a complete infectious cDNA clone of HAV was constructed. Initially, HAV cDNA clones from wild-type HAV (HM-175) were ligated together to produce a full length copy of the HAV genome in pBR322. The entire cDNA construct was sequenced and no changes were detected between this construct and its parent cDNA clones. Transfection of marmosets with this cDNA failed to initiate HAV infection. The HAV insert was excised from pBR322 and inserted without the oligo (dG)·oligo (dC) tails into an RNA transcription vector. Transfection of marmosets with the resulting plus strand RNA also failed to initiate HAV infection. An attenuated, cell culture-adapted mutant of HAV (passage 32) was then molecularly cloned. Three overlapping cDNA clones were obtained which together spanned the entire genome. These clones were ligated together to produce a full length cDNA copy of the attenuated HAV in pBR322. Transfection of monkey kidney cells with this plasmid failed to yield HAV. The HAV cDNA was then excised and inserted without its oligo (dG)·oligo (dC) tails into an RNA transcription vector. Transfection of monkey kidney cells with this cDNA induced HAV infection. Transfection with RNA transcripts produced in vitro from the cDNA yielded about 10-fold more HAV than transfection with cDNA. The infectious HAV cDNA retained its attenuation phenotype during infection of marmosets (Cohen, Ticehurst, Feinstone, Purcell).

Chimeric cDNAs which contain portions of both wild-type and attenuated HAV cDNA were recently constructed. The HAV genome of each virus was divided into three portions-- (a) 5' noncoding region, (b) P1 (capsid region), and (c) P2-P3 (nonstructural proteins plus 3' noncoding region), and all six possible combinations of wild-type and attenuated fragments were constructed. RNA transcripts from three of the cDNA constructs were infectious when transfected into monkey kidney cell culture. Lysates from cells transfected with the three cDNAs will be inoculated into marmosets to determine which portion(s) of the genome is (are) associated with attenuation. RNA transcripts from the other three chimeric cDNAs were not infectious. In each case cDNA in the P2-P3 and 3' noncoding region originated from wild-type virus. This suggests that the wild-type cDNA is not infectious due to a mutation which developed in this region during cloning, or that mutations in this region are responsible for cell culture-adaptation and as a consequence virus with wild-type sequence in this region grows poorly in cell culture. Studies employing infectious chimeric cDNA and site-directed mutagenesis should allow us to define the mutations in the tissue culture adapted HAV that are responsible for attenuation (Cohen, Feinstone, Purcell).

Hepatitis B Virus (HBV): Vaccinia Virus - HBsAg Recombinant. Hepatitis B vaccines have been developed and are currently licensed in a number of countries throughout the world. These consist of the surface antigen of the virus (hepatitis B surface antigen, HBsAg) purified from the plasma of chronic HBsAg carriers, inactivated with formalin or heat and adsorbed to alum. Such vaccines have been shown to be safe, immunogenic and efficacious in field trials. Their chief disadvantages are that they are very expensive, available in limited quantities, and, until recently, required safety-testing in chimpanzees, the only susceptible animal suitable for such tests. An intriguing application of DNA recombinant technology to the development of second-generation vaccines involves the construction of vaccinia virus recombinants pioneered by Dr. B. Moss of LVD. A 1350 bp fragment of HBV DNA that contains the coding sequence for HBsAg was inserted into the TK gene of vaccinia virus. The HBsAg gene was placed under the control of the 7.5 early-late promoter of vaccinia virus. In collaborative studies with Dr. Moss, chimpanzees infected intradermally with the vaccinia-HBsAg recombinant did not develop demonstrable HBsAg antibodies but they were protected against hepatitis when subsquently challenged with live HBV. These chimpanzees developed a high titer of HBsAg antibodies at a time after challenge when HBsAg was first detected in the plasma of a control, unvaccinated chimpanzee, suggesting that the vaccinated animals had been "primed" by infection with the vaccinia recombinant and developed an anamnestic antibody response to newly-synthesized HBsAg following challenge with live HBV. This experiment was repeated with two modifications. First, the recombinant vaccinia virus used for vaccination was constructed from a vaccinia virus strain used for production of vaccine licensed for human use rather than the more virulent laboratory strain used in the first experiment. Second, the two immunized chimpanzees were revaccinated with the vaccinia recombinant one month after the primary vaccination in an attempt to "boost" the immune response, a procedure that yielded high antibody titers in small laboratory animals. The chimpanzees were challenged with live HBV and followed for evidence of infection. Neither chimpanzee developed detectable HBsAg antibodies after either vaccination. When the chimpanzees were challenged with live HBV, both developed typical type B hepatitis, indicating that vaccination with the recombinant vaccinia strain failed to stimulate resistance. More efficient expression of the HBsAg gene in vaccinia must be engineered before this approach will have practical application (Purcell).

Pre-S2 Peptide Immunization. A region of the HBV genome other than that coding for the S (surface antigen) protein may also play a role in immunity to HBV. The "pre-S" region, which immediately precedes the S gene contains two domains (pre-S1 and pre-S2) that code for a series of peptides that are actually "fusion" proteins consisting of the gene product of part of the pre-S region followed by the complete S region. These fusion proteins, ranging in size from approximately 33 kd to 46 kd, are minority components of HBsAg but appear to be integral parts of the virion, and some of them are present on the surface of the virion. The pre-S2 region of one of these peptides contains a receptor site for polymerized human serum albumin, and this receptor is thought to be important in binding of HBV to hepatocytes. Antibodies to epitopes found in the pre-S2 region are present in the serum of most individuals convalescing from HBV, and pre-S2 antibodies appear to be the first HBV antibodies that can be detected in many infected individuals. To examine this further, chimpanzees were immunized with synthetic peptides representing a portion of the pre-S2 gene product. In this study, carried out collaboratively with Drs. R. Lerner and J. Gerin, chimpanzees responded with rapid development of large amounts of antibody to the pre-S2 region; this was in contrast to the poor antibody response seen previously with synthetic peptides representing portions of the S gene product. These chimpanzees were challenged to determine if the gene product of the pre-S2 region alone could protect against HBV. At the time of challenge both animals still possessed antibody to the pre-S2 region as measured by radioimmunoassay (RIA) with synthetic pre-S2 peptides. However, only one of the animals still possessed antibodies that reacted with native pre-S by RIA. This chimpanzee was completely protected from challenge; the other chimpanzee was partially protected but developed a mild hepatitis. These studies, coupled with the results of a similar protection study of synthetic pre-S2 peptides in Japan, indicate that the pre-S2 region of HBsAg also contains an epitope that stimulates protective antibody (Purcell).

However, preliminary analysis of serial serum samples from the infants who were protected from transmission of maternal HBV infection during our recent HBsAg vaccine efficacy trial in China revealed that pre-S2 antibodies appeared in only a few of the infants vaccinated with either the NIH or Chinese vaccine and that, when such antibodies did develop, they generally appeared late, i.e., after six months. Since infants of chronically infected mothers usually become antigenemic within the first three months, such late appearing antibodies would probably not be protective. These data suggest that antibodies to the pre-S2 epitopes may not be required for prevention of type B hepatitis (Purcell).

The current intense interest in developing vaccines for AIDS has prompted a reevaluation of the extensive experience we and others have had in developing hepatitis B vaccines. It is now clear that a successful HBV vaccine must be capable of stimulating antibody to native viral antigen. Furthermore, the antibody must be long-lived and not just transient. To date, only HBsAg expressed in a suitable eukaryotic expression system (yeast, mammalian cells) has proven to be suitable for a practical vaccine. Other methods, such as vaccination with recombinant vaccinia virus, synthetic peptides or anti-idiotype antibodies, do not appear to have much potential for vaccine development in their present form. The implications for AIDS vaccine development are obvious (Purcell).

HBV Protease. Recent observations suggest that hepatitis B virus, a member of the hepadnavirus family, is evolutionarily related to the retroviruses. Available evidence indicates that, like the retroviruses, HBV may synthesize its polymerase protein as a nucleocapsid-polymerase fusion polyprotein. Although a protease is required to cleave the fusion polyprotein to release an active polymerase, such a protease activity or viral protease-like gene sequence has not been described in hepadnaviruses. Using computer analysis, an amino acid sequence at the amino terminus of the core protein sequence was identified which is homologous to the protease gene sequence of retroviruses. The putative hepadnavirus protease, in addition to processing the nucleocapsid-polymerase polyprotein, may produce HBeAg through proteolytic self-cleavage of the core protein. This would explain the existing experimental data on the labile nature of the HBcAg (core) protein in eukaryotic expression systems as well as the origin of HBeAg in infected hepatocytes (Miller).

Woodchuck Hepatitis Virus (WHV). The woodchuck hepatitis virus (WHV) is taxonomically and serologically related to the hepatitis B virus (HBV). Infection with each of these viruses is associated etiologically with acute and chronic hepatitis and hepatic cell carcinoma in their respective hosts. The WHV/woodchuck model system provides a convenient means of studying the relationship between virus and host in the oncogenic process. The WHV and HBV genomes contain four long open reading frames encoding the viral core, polymerase, and surface proteins, as well as a protein with an unknown function (X). Recent studies by other laboratories suggest that the X gene protein of hepadnaviruses, like the tat gene protein of HIV, may be responsible for transactivation of viral gene expression. Hence, experiments were performed to determine whether X gene sequences are essential for virus replication. Four recombinant DNA molecules of the WHV genome were constructed: an infectious WHV wild type genome (positive control), a deletion mutant lacking the carboxyl (C) terminal end of the X gene, a deletion mutant lacking both the C and amino (N) terminal ends of the X gene, and a deletion mutant lacking the entire 3' end of the WHV genome (negative control). The constructs were amplified in bacteria, and viral DNA sequences were purified from bacterial sequences and ligated under conditions optimal for formation of monomeric circular molecules. The recombinant DNA molecules were transfected directly into the liver of adult woodchucks using the calcium phosphate precipitation method. The animals were bled weekly and serum was assayed for the appearance of the viral surface protein as an indication of viral replication. Recombinants lacking full length sequences of the X gene did not initiate infection suggesting that the deleted X gene sequences are essential for virus replication (Miller).

The complete nucleotide sequence of virion DNA from two isolates of woodchuck hepatitis virus (WHV 7 and WHV 59) was determined. The sequences of the two WHV isolates were compared with the previously published sequences of two other isolates (WHV 1 and WHV 8). The range of nucleotide sequence variation of the four isolates (1 to 3.5%) was similar to that of HBVs of the same subtype (1.5 to 2%), but less than that of HBVs of different subtypes (8 to 10%). Analysis of overlapping genes in WHV indicates that different portions of the polymerase and X genes seem to evolve independently and at different rates. The overlapping portions of these genes appear to evolve at a faster rate (with more nucleotide and amino acid variation) than the nonoverlapping portions, despite the constraints imposed by having to code for two different proteins (Cohen, Miller).

Blood Borne Non A, Non B Hepatitis Viruses (NANB). The agent of blood borne non-A, non-B hepatitis continues to resist all attempts at identification. A selected subtractive cDNA library was produced and screened for specific NANB hepatitis virus or disease related sequences, but none were identified. A second more highly selected library is presently being constructed. A cDNA expression library in λ gt 11 was constructed and screened with Dr. Shimizu's NANB monoclonal antibody which reacts with an antigen in NANB virus infected liver. Clones producing an antigen that reacted with the Shimizu antibodies were not isolated. The relationship of the NANB agent to other viruses, especially HBV and HDV, is being investigated using the primer pair amplification system developed at Cetus Corp. (Feinstone).

Inactivation of Hepatitis Viruses in Pooled Plasma Derivatives. The occurrence of viral hepatitis in recipients of blood products, especially pooled plasma derivatives, continues to be a major problem. Mandated screening of all plasma units for HBsAg has diminished (but not abolished) type B hepatitis in hemophiliacs and other recipients of clotting factors but NANB hepatitis continues to be a major medical problem for these individuals. In addition, the high prevalence of acquired immune deficiency syndrome (AIDS) in hemophiliacs and the fact that over half of such individuals are infected with human immunodeficiency virus (HIV), the etiologic agent of AIDS, adds urgency to the development of methods that can eliminate hepatitis viruses and HIV from clotting factors without markedly diminishing the potency of these labile proteins. Chloroform is now being evaluated for its ability to inactivate HBV, NANB and HIV because each of these viruses possesses essential lipids, i.e., these viruses are inactivated by lipid solvents. Partially saturated chloroform was found to be partially effective and fully saturated chloroform fully effective in inactivating other enveloped, lipid-containing viruses, even though, saturated, the chloroform contained less than 1% water and could be handled as a single liquid phase. This procedure, when applied to commercial lyophilized factor VIII, results in retention of 100% of the factor VIII activity, in contrast to extraction of aqueous factor VIII with chloroform, which results in a large and variable destruction of factor VIII activity, depending on its purity and method of preparation.

NANB introduced experimentally into factor VIII was completely inactivated by chloroform treatment of lyophilized material. Similar inactivation studies of HIV were carried out in collaboration with Dr. Gerald Quinnan (FDA). HIV was introduced into factor VIII and, following lyophilization, the factor VIII was exposed to a number of different inactivation procedures, including heat, water-saturated chloroform and a number of chemical inactivating agents. Dry heat, which is currently used for a number of commercial preparations, inactivated HIV but not as efficiently as the water-saturated chloroform and a number of chemical inactivating agents. These studies indicate that chloroform is potentially useful for the inactivation of lipid-containing viruses in plasma products and that it may be used either as a two-phase water/chloroform preparation for treating plasma products in solution or as a water-saturated single-phase chloroform preparation for treatment of lyophilized products (Feinstone, Purcell).

Because Bradley et al., described two NANB hepatitis viruses in clotting factors VIII and IX, one that is sensitive to chloroform and one that is not, the same lots of clotting factor studied by Bradley were reexamined in LID. Our analysis indicated that only one chloroform-sensitive NANB virus was present in these lots of the clotting factors (Feinstone, Purcell).

Epidemic Non A, Non B Hepatitis Viruses. In earlier collaborative studies with scientists in India and Algeria, outbreaks of epidemic water-borne NANB hepatitis as well as clusters of cases of endemic NANB hepatitis were identified and analyzed. These outbreaks occurred in Delhi, Ahmedabad, Pune, Kolhapur, and Algiers between 1955 and 1985. None of the outbreaks could be related serologically to infection with HAV or HBV, although the pattern of disease closely resembled that of type A hepatitis. These outbreaks of hepatitis appeared to be caused by an agent serologically distinct from currently recognized HAV strains. The etiologic agent may represent a previously unrecognized serotype of HAV or an entirely different agent (Purcell).

Attempts to transmit an agent to chimpanzees and marmosets using clinical specimens from some of these outbreaks of epidemic/endemic non-A non-B hepatitis (ENANB) were partially successful. Transient low-level liver enzyme elevations and histopathologic changes in liver biopsies consistent with hepatitis were observed in some animals, but this was not uniform, and attempts to transmit an agent serially also produced irregular results (Purcell, Ticehurst).

Although an etiologic agent was not identified in experimental animals and none of the specimens from the outbreaks hybridized with probes that detect a wide range of picornaviruses, including HAV, several different virus-like particles have been visualized. One of these particles, present in feces from an Indian patient in Amedabad, is approximately 27 nm in diameter and appears to have surface structure. This particle apparently has a wide geographic distribution because it reacts with sera from Algeria, USSR, Pakistan and India. A naturally infected human and an experimentally inoculated chimpanzee developed a serologic response to the particle as demonstrated by immune electron microscopy (IEM). This virus appears to be the most promising candidate of the many putative etiologic agents of ENANB hepatitis (Ticehurst, Purcell, Kapikian).

Hepatitis Delta Virus (HDV). The delta virus is a transmissible hepatitis virus that is defective and requires co-infection with HBV for its own synthesis. The agent has a small RNA genome ($10^{5.7}$ daltons) that is encapsidated together with delta antigen within a coat of HBsAg. Evidence for infection with the delta agent is found most frequently in carriers of hepatitis B virus who are repeatedly exposed to blood (hemophiliacs, illicit drug users, etc.). Sensitive assays for delta agent have been developed and used to evaluate experimental infection of HBV-carrier chimpanzees. In both humans and chimpanzees, superinfection with the delta agent causes very severe hepatitis.

In collaboration with the Chiron Corp., Dr. J. Gerin (Georgetown University), has cloned the genome of the delta agent. Analysis of the HDV genome revealed it to be a negative strand virus with up to five open reading frames (ORF's). However, only one of these is known to code for a protein (delta antigen, HDAG). The genome of HDV contains certain sequences that are highly conserved among viroids of plants. These conserved sequences are also found in certain forms of introns, suggesting that both the plant pathogens and HDV may have originated from eukaryotic cells. Delta cDNA has been used as a probe for the detection of HDV RNA in clinical samples. HDV RNA was found in over half of patients with either acute or chronic HDV infection and its detection correlated well with other markers of HDV infection, including HDAG in liver biopsies and IgM anti-HD in serum. Thus, HDV RNA provides a specific marker of active HDV replication (Purcell).

In earlier experiments, HBV carrier chimpanzees rechallenged with the HDV were resistant to reinfection when the challenge was administered approximately six months after the initial delta hepatitis. However, in recent experiments, we have had the opportunity to rechallenge chimpanzees 3 to 5 years after their initial delta infection. Three of 4 rechallenged chimpanzees developed definite hepatitis. It was not possible to demonstrate that these illnesses were the result of replication of either HDV or HBV. An additional HBV carrier chimpanzee which had never been inoculated with either HDV or NANB hepatitis virus was inoculated with plasma samples from the rechallenged animals. This chimpanzee developed typical delta hepatitis, indicating that "rechallenge hepatitis" was caused by limited replication of HDV in previously infected animals. This finding has implications for the interpretation of hepatitis occurring among human HBV carriers. Although some of these illnesses have been identified as due to superinfection by HDV, others are thought to be caused by NANB hepatitis virus, based upon the absence of serologic response to HDV. This will now have to be reevaluated (Purcell).

Preliminary hybridization studies with subgenomic cDNA from HDV suggested that HDV infection in chimpanzees usually was self-limited. However, recent hybridization studies with probes consisting of strand-specific RNAs transcribed *in vitro* that represent the entire genome indicate that many infections of chimpanzees in fact become chronic. Thus, experimental infection of chimpanzees resembles chronic infection of man more closely than was previously appreciated.

Transmission of HDV to woodchucks chronically infected with the woodchuck hepatitis virus was observed in previous studies with Dr. A. Ponzetto (Turin, Italy) indicating that this hepadnavirus can also serve as a helper for HDV replication. Dr. Ponzetto has serially passaged HDV in chronic WHV carrier woodchucks and the fifth woodchuck passage HDV was inoculated into an HBV carrier chimpanzee. This animal developed hepatitis, indicating that the HDV had not lost its ability to replicate in primates.

Dr. Yohko Shimizu has generated monoclonal antibodies that react with a cytoplasmic antigen in hepatocytes from chimpanzees experimentally infected with NANB hepatitis virus. In collaborative studies with Dr. Shimizu it was shown that a presumably identical antigen is present in hepatocytes from chimpanzees experimentally infected with HDV. Extensive evaluation of experimentally infected chimpanzees confirms that this antigen is not the result of contamination of the HDV inoculum with NANBV. Instead, it is an antigen, possibly of host origin, that is induced by infection with either NANBV or HDV (Purcell).

Respiratory Viruses

Respiratory Syncytial Virus (RSV) Genome. RSV is an enveloped RNA-containing virus that is the most important cause of pediatric respiratory tract disease. The genome of RSV (νRNA) is a single negative strand of RNA of approximately 15,000 nucleotides. Previously, analysis of cDNA clones of intracellular viral mRNAs of RSV strain A2 established that the viral genome encodes ten mRNAs and provided identification of ten major viral proteins. RSV encodes a major nucleocapsid (N) protein, a nucleocapsid-associated phosphoprotein (P), an envelope matrix protein (M), a second, unrelated matrix protein (22K), a large (L) nucleocapsid protein (polymerase), two envelope glycoproteins (F and G), two nonstructural proteins (1C and 1B) and a small hydrophobic protein (1A). Complete nucleotide sequences have been determined for 9 of the 10 genes (excepting the L gene). Only the genomic termini and the distal region of the L gene remain to be sequenced. Sequence analysis of intergenic and flanking gene sequences identified the viral gene order (3' 1C-1B-N-P-M-1A-G-F-22K-L) as well as the sequences of the intergenic regions (annual report, 1986). Mapping and sequencing of the 5' end of the L mRNA showed that the L gene overlaps with its upstream neighbor, the 22K gene (annual report, 1986) (Collins, Spriggs, and Olmsted).

There are 2 antigenic subgroups of RSV, subgroup A and subgroup B. Because the A2 strain, which belongs in subgroup A, had been analyzed extensively it was of interest to study the 18537 strain of RSV, which belongs in subgroup B, and compare its viral genes and genome structure to those of a subgroup A virus. cDNAs for the 18537 strain were generated for 9 of the 10 viral genes (excluding the L gene). Comparison with the A2 sequences showed that the gene order was identical to that of the subgroup A strain and the deduced amino acid sequences and nucleotide sequences of open reading frames were highly conserved (76-96% identity at the amino acid level) except for the G gene and its protein (53% at the amino acid level). Putative transcriptional signals were also highly conserved while noncoding gene and intergenic sequences exhibited little conservation. The 68 nucleotide overlap of the 22K gene (5' non-coding sequences in vRNA sense) and the L gene (L gene-start signal followed by translational start codon for the L ORF) was almost completely conserved (92% identity), while the position of the translational start site for the L ORF was conserved exactly. The putative extracellular domain of the G glycoprotein exhibited little conservation (44% identity) except for a short circumscribed region that was highly conserved in sequence. This region, which contains 4 conserved cysteine residues, is suspected of playing a role in binding virus to host cell receptors. The sequence heterogeneity in the extracellular domain of the G glycoprotein could well be a mechanism for evading host immunity. The ability of G to sustain extensive and rapid (relative to the other RSV proteins) amino acid substitutions without loss of function suggests that its higher order structure might be relatively flexible and simple (Johnson, Collins).

RSV - Processing of Viral Proteins. The structure and processing of the small 1A protein and the attachment (G) glycoprotein of human RSV were investigated using recombinant viral vectors, site-directed mutagenesis and peptide-specific antisera. The 1A protein was shown to be a third integral membrane protein of RSV infected cells. The 1A protein is processed intracellularly by a multi-step pathway that includes glycosylation, and it is displayed in abundance at the cell surface. Although the 1A protein is present at the surface of infected cells it does not appear to play a demonstrable role in immunity to RSV. A vaccinia virus-1A recombinant did not induce resistance to RSV in cotton rats nor did recombinant infected cells serve as targets for RSV cytotoxic T cells (Olmsted, Collins).

Site directed mutagenesis was used to construct a series of G protein mutants that contained COOH-terminal truncations of increasing length. Expression of these mutants in a late-replacement SV40 vector permitted us to map protein domains that are involved in the addition of O-linked sugar and in cell-surface transport. Of interest was the observation that the signals for transport to the cell surface and for O-glycosylation were contained within the first 71 and 180 amino acids, respectively, of this heavily O-glycosylated glycoprotein which is 298 amino acids in length. These G mutants may prove useful in mapping antigenic sites (Collins, Olmsted).

RSV - Viral Proteins and Their Role in Immunity. Antigenic sites on the fusion (F) glycoprotein were identified using a panel of 19 monoclonal antibodies (mAbs), 11 of which neutralized viral infectivity. Some of the neutralizing mAbs (N-mAbs) exhibited enhanced neutralizing activity in the presence of complement and one mAb required complement for virus neutralization. Most N-mAbs also inhibited fusion activity, but there was no strict correlation between high neutralizing activity and high fusion-inhibiting activity. The panel of N-mAbs recognized a minimum of 4 distinct epitopes, which appeared to be distributed within 3 antigenic sites. The total number of F epitopes recognized by these N-mAbs will be known when analyses of their reactivity patterns with laboratory-selected monoclonal antibody-resistant mutants (MARMs) and RSV clinical strains is completed. Several of the MARMs are also phenotypic variants, which produce a lytic form of cytopathology instead of the usual syncytium-formation. Identification of the amino acid substitutions responsible for these phenotypes should advance our understanding of the relationship between the molecular structure of the F glycoprotein and its biological functions (Beeler, Coelingh).

The antigenic relationship between the F and G glycoproteins of the two RSV antigenic subgroups was studied using RSV-vaccinia virus recombinants and glycoprotein-specific ELISA. These studies showed that the F glycoprotein was highly conserved antigenically, whereas the G glycoprotein exhibited significant antigenic divergence. This underscored the importance of the F glycoprotein in any RSV vaccine strategy. These findings, together with the observation that the F glycoprotein induces significantly greater immunity than the G glycoprotein (annual report, 1986), suggests that the F glycoprotein alone might fulfill the minimum requirements for an RSV vaccine. Although the G glycoprotein exhibited significant antigenic divergence, it did induce substantial heterologous resistance in cotton rats immunized with a vaccinia-RSV G recombinant. In fact, the degree of heterologous protection was surprisingly high considering the extensive amino acid sequence divergence of the extracellular domain of G between subgroups (44% identity) as well as apparent extensive differences in the location of O-linked and N-linked carbohydrate side-chains. The antigenic dimorphism of the G glycoprotein suggests that it may be advisable to include the G glycoprotein of both subgroups in any RSV vaccine designed to induce immunity to this viral antigen. Towards that end, site-directed mutagenesis has been used to engineer the 18537 G cDNA for insertion into expression vectors (Johnson, Collins, Olmsted).

Recombinant RSV-vaccinia viruses were also constructed for the 1C, 1B, N, P, M, 1A and 22K proteins to investigate processing, function, and contribution to host immunity of the individual RSV proteins. In collaboration with Dr. J. Yewdell (Wistar Institute) the F and N proteins, but not the 1A and G proteins, were shown to be major targets for RSV-specific murine cytotoxic T cells. Immunization of cotton rats with a vaccinia virus recombinant expressing the 1A or N protein did not induce significant resistance to RSV infection. The F and G glycoproteins have also been expressed in insect cells by recombinant baculoviruses for possible use in a subunit vaccine and as an additional system for investigation of protein processing (Collins, Olmsted).

RSV - Subgroup Antigenic Divergence. Analysis of the two antigenic subgroups of RSV described previously was extended by characterizing the RSV subgroup-specific antibody response of infants to primary infection with RSV. Homologous and heterologous responses to the F glycoprotein were essentially similar. In contrast, heterologous antibody responses to the G glycoprotein were significantly reduced; Archetti-Horsfall analysis indicated a 5% relatedness between the G glycoproteins of subgroups A and B. Neutralizing antibody responses to homologous and heterologous viruses were also significantly different, although to a lesser extent; the two subgroups were approximately 30% related by neutralization assay. These data indicate: (i) that two naturally occurring subgroups of RSV are seen by the human immune system during primary infection as antigenically distinct but moderately related viruses; (ii) that RSV antigenic dimorphism may make a contribution to the susceptibility of infants and children to reinfection with RSV; (iii) that RSV antigenic dimorphism should be taken into account when formulating a strategy for vaccination against RSV infection (Murphy, Chanock).

RSV - Mechanism of Disease Potentiation by Formalin-Inactivated Vaccine. Potentiation of RSV disease was observed in cotton rats (*Sigmodon hispidus*) previously immunized with formalin-inactivated RSV. This potentiation of disease resembled that observed 20 years ago during a clinical trial of formalin-inactivated RSV vaccine in young infants. Animals immunized with the Lot 100 RSV vaccine used in the clinical trials of 20 years ago, or with freshly-prepared, formalin-killed RSV, were challenged intranasally with RSV. These animals developed an accentuated pathological response to infection. This potentiated response was characterized initially by polymorphonuclear infiltration of the lungs which was followed by an infiltration of lymphocytes; the former began one day after viral challenge. Further analysis of this phenomenon indicated that IgG and C3 were deposited in both the lungs and kidneys. Also, pulmonary pathology was less severe in complement-depleted animals. Significantly, a dose-response effect was observed which was similar to that described for experimental immune complex disease. These observations suggested that the potentiated disease caused by formalin-inactivated RSV vaccine was in part the result of a pulmonary Arthus reaction (Prince, Murphy, Chanock).

In infants and young children formalin-inactivated RSV vaccine induced a high level of binding (ELISA) antibodies to the RSV F glycoprotein but these antibodies were deficient in both neutralizing and fusion-inhibiting activities. Radioimmunoprecipitation of post-immunization sera revealed that antibodies to the internal viral M and NP antigens were also produced by a majority of the vaccinees tested. Thus, formalin-inactivated vaccine also induced high levels of antibodies to RSV M and NP proteins, antigens which are not involved in protective immunity. These antibodies as well as the non-functional antibodies to the viral surface glycoproteins may have acted in concert to produce a pulmonary Arthus reaction which contributed significantly to the potentiation of disease that occurred when vaccinees were infected with RSV (Murphy, Chanock).

RSV - Immunotherapy. In collaboration with Dr. Val Hemming (USUHS) and Dr. William Rodriguez (Childrens Hospital National Medical Center) a double-blind, placebo-controlled intravenous immunoglobulin (IVIG) immunotherapy study was performed in 35 infants hospitalized with RSV disease. The treatment was well tolerated and resulted in a significant reduction in level of RSV shed from the upper respiratory tract as well as a significant improvement in transcutaneous oximetry readings. However, the mean duration of hospitalization was not reduced by IVIG treatment. To date, follow-up has not revealed any harmful effects resulting from immunotherapy of RSV illness (Prince, Chanock).

In studies performed in cotton rats, IVIG administered topically offered a significant advantage over systemically administered IVIG, effecting near-complete clearance of pulmonary RSV at a dose 160-fold less than that required for the same therapeutic effect with parenterally administered IVIG. The therapeutic effect of topical IVIG was long lasting, and was not due to *in vitro* viral neutralization during homogenization of pulmonary tissue. Topically administered IVIG, when given prophylactically, provided significant resistance to pulmonary RSV infection for up to seven days, suggesting that there is a specific receptor for IgG in the lungs. A similar therapeutic effect of IVIG administered topically into the respiratory tract was observed in monkeys. The therapeutic effect of topically administered IVIG appears to be mediated within the lumen of the lungs rather than within the parenchyma, thus underscoring the importance of local surface immunity. The quantity of IVIG present in the serum of topically treated cotton rats which received the minimum dose required for the almost complete clearance of RSV from lung tissues (i.e., 0.025 g/kg) was 61 ug/ml. This level was 200 times lower than the 12,379 ug of IVIG per ml which was present in the serum of animals treated parenterally with the minimum dose required for the almost complete clearance of RSV from lung tissues (i.e., 4g/kg). Thus, the amount of IVIG in the serum of infected cotton rats successfully treated topically with immunoglobulin was considerably below the concentration of IVIG associated with a full therapeutic effect in parenterally treated animals, implying that IVIG exerts its therapeutic effect within the air passages of the lungs, presumably on cells that line these passages (Prince, Chanock).

RSV - Experimental Immunoprophylaxis With Vaccinia Virus Recombinants.

Previously it had been shown a vaccinia virus-RSV F or a vaccinia virus-RSV G recombinant or both together induced significant resistance to infection with RSV in the lower respiratory tract of cotton rats. The vaccinia virus used to construct these recombinants was the WR strain which is a rodent adapted, neurovirulent mutant that is clearly unsuitable for use in humans. The use of this virulent mutant for preparation of recombinants did, however, offer an opportunity to evaluate the effect on virulence of insertion of a foreign gene into the TK gene of vaccinia virus. Previously, Dr. Mark Buller (LVD, NIAID) showed that the virulence of vaccinia virus in mice was reduced by insertional inactivation of the TK gene during the construction of recombinant viruses. However, the mouse model was not optimal because this animal is resistant to vaccinia virus and because an atypical route of administration (intracranial) was required to induce fatal disease. During our studies it became apparent that the cotton rat offered a more sensitive system for comparing the safety of recombinant viruses to that of their parental vaccinia strain. Intradermal (ID) inoculation of cotton rats with the parental (strain WR) vaccinia virus resulted in systemic virus dissemination, significant pathology in the liver, and significant mortality. Animals inoculated with 10^5 pfu had a 20% mortality, and a dose of 10^6 pfu and greater resulted in 80% mortality. Overall, the LD_{50} was estimated to be less than 10^4 pfu. In contrast, TK^- recombinant viruses, such as vaccinia-F, did not kill any animals even when administered at a dose of $10^{8.7}$ pfu. The LD_{50} for the TK^- recombinants could not be determined because of their highly reduced virulence, but it is at least 10^5 greater than the LD_{50} for the parental strain. Therefore, insertional inactivation of the TK gene resulted in a 10^5 or greater reduction in virulence of the WR strain of vaccinia for cotton rats. These observations are encouraging, but on the other hand reduction in replicative capacity of vaccinia virus recombinants prepared from a more temperate strain suitable for use in humans might result in overattenuation and an unacceptable decrease in immunogenicity. This issue can only be resolved by studies which are now in progress (Olmsted, Collins, Murphy, Chanock).

Recently completed studies in four species of lower primates indicated that the vaccinia-RSV F and vaccinia-RSV G recombinants prepared from the WR strain were satisfactorily immunogenic in 2 of the species; rhesus and patas monkeys developed a level of serum RSV neutralizing antibodies that had previously been associated with resistance to RSV in the lower respiratory tract of infants and cotton rats. These observations indicate that some lower primates develop a protective immunologic response to RSV following infection with vaccinia-RSV recombinants. However, the size of intradermal lesions induced by the recombinants was 5-15 times smaller than that of lesions induced by the parental WR vaccinia virus indicating that insertion of either RSV glycoprotein gene into the TK locus of vaccinia virus attenuates its replicative capacity in the skin of primates (Olmsted, Collins, Murphy).

Currently two strains of vaccinia virus which have been used extensively in human immunoprophylaxis are being evaluated as parents for recombinants to be employed in human vaccines.

Human Parainfluenza Virus Type 3 (PIV3): Genome and Viral Proteins. Human parainfluenza virus type 3 (PIV3 virus), a paramyxovirus, is second in importance only to RSV as an etiologic agent of pediatric respiratory tract disease. Previously, PIV3 was shown to encode six structural proteins: the HN (69-70 Kdal) and F (60-63 Kdal) glycoproteins, which constitute the virion envelope spikes; the M protein (35-40 Kdal) which underlies the lipid envelope; and the NP (66-68 Kdal), P (83-90 Ddal) and L (200 Kdal) proteins, which associate with νRNA to form the nucleocapsid. PIV3 also has a single non-structural protein C (22 Kdal) that was shown to be encoded by a second open reading frame in the P+C mRNA. We previously constructed cDNA clones of 5 of the 6 viral mRNAs (excluding only L mRNA), identified their encoded protein products, determined the viral gene order and sequences of intergenic regions of viral genomic RNA (vRNA) as well as sequences for the NP, P+C, F and HN mRNAs. cDNAs of the F and HN mRNAs were then engineered by site-directed mutagenesis for insertion into recombinant vectors (Spriggs, Collins).

Amino acid sequence homologies and similarities in gene map were examined to investigate relationships between PIV3 and other paramyxoviruses. These comparisons provided evidence for a close evolutionary relationship between PIV3 and Sendai virus (murine parainfluenza virus type 1) and a more distant relationship with simian virus 5 (canine parainfluenza virus type 2) (Spriggs, Collins).

Recombinant vaccinia viruses were constructed that expressed F, HN or NP protein that appeared to be authentic biochemically, biologically and immunologically. Immunization of cotton rats with either vaccinia-F or vaccinia-HN or both induced a high level of PIV3-specific serum neutralizing antibodies and immunized animals exhibited significant resistance to challenge with PIV3. This established that each of the glycoproteins is a major neutralization antigen, although HN appeared to play the greater role in protective immunity. Immunization of nonhuman primates (patas monkeys) with vaccinia F and vaccinia HN induced a high level of serum neutralizing antibodies and immunized animals exhibited significant resistance to PIV3 challenge in both the upper and lower respiratory tract. This was the first demonstration that a single parenteral inoculation of a recombinant virus can induce immunity to a respiratory tract virus in primates. Also, the HN cDNA was expressed in biologically active form with a late-replacement SV40 vector. Site directed mutagenesis was used to investigate the role of specific polypeptide domains and amino acid residues in intracellular transport of HN and in neuraminidase and hemagglutination activities (Spriggs, Collins).

Human PIV3 Hemagglutinin-Neuraminidase (HN) Glycoprotein. Previous antigenic analyses of human PIV3 clinical strains isolated over a 27-year period in three widely separated geographic regions indicated that variation occurred in approximately half of the detectable HN epitopes. The molecular epidemiology of PIV3 was investigated further by sequencing the HN gene of six clinical strains isolated between 1973 and 1983 comparing their nucleotide and predicted amino acid sequences with those of the prototype human PIV3 strain recovered in 1957. The HN genes (1716 nucleotides) of the six clinical strains differed from that of the prototype strain at a total of 164 nucleotide positions. This variability did not result from an accumulation of mutations over time, but rather represented genetic heterogeneity in the HN genes of various PIV3 strains. Most of the nucleotide diversity occurred in the 5' non-coding sequences, exclusive of regions previously identified as transcriptional and translational control elements. Although the overall deduced amino acid homology among HN proteins was high (96.7-99.1%), most variability was concentrated in domains at the amino and carboxyl termini of the HN protein. The low overall rate of change in the PIV3 HN probably reflects the minor selective pressures encountered by this virus in nature, since PIV3 infection of humans typically induces only partial immunity (Coelingh).

Baculovirus recombinants expressing PIV3 HN protein were constructed in order to expand our previous antigenic and functional characterization of this viral glycoprotein to include structural analysis. The HN protein produced by these recombinants in insect cells was glycosylated, transported to the cell surface, biologically active, immunogenic, and antigenically authentic. These properties indicate that recombinant-derived HN should be a suitable substrate for structural analysis by x-ray crystallography. In addition, this material may prove to be a useful immunogen for vaccination against human PIV3 because cotton rats immunized with a lysate of baculovirus-PIV3 HN recombinant infected insect cells exhibited significant resistance to challenge with human PIV3 (Coelingh).

Bovine PIV3 As a Candidate Vaccine Strain For Immunization Against Human PIV3. Human and bovine PIV3 HN proteins share three epitopes in antigenic site A (recognized by neutralizing mAbs) and two epitopes in antigenic sites D and F (recognized by non-neutralizing mAbs). The remaining nine human PIV3 HN epitopes are not detected on the bovine PIV3, presumably reflecting evolutionary divergence of these viruses in different hosts. Although the bovine PIV3 is clearly different from human strains, it does share common HN neutralization epitopes and is able to induce resistance to human PIV3 in cotton rats. For these reasons bovine PIV3 was evaluated further in primates to determine its potential as a live virus vaccine for prevention of human PIV3 disease. Infection of squirrel monkeys with bovine PIV3 induced a moderate immune response to human PIV3 and these animals exhibited resistance to subsequent challenge with human PIV3. Although all of the immunized monkeys became infected with human PIV3 after challenge, the duration of viral shedding was reduced markedly and the level of viral replication in the lower respiratory tract was reduced 1000-fold. Evidence of attenuation of bovine PIV3 for primates was then sought in rhesus monkeys and chimpanzees. In chimpanzees, prototype human and bovine PIV3 strains grew to the same titer in the upper respiratory tract (10^5 $TCID_{50}$/ml) and the duration of viral shedding was the same, 10 days. In the lower respiratory tract, however, replication of the bovine strain was restricted 100-fold. In rhesus monkeys replication of the prototype bovine PIV3 and a recent bovine isolate was reduced 100-fold in the upper respiratory tract and 1000-fold in the lower respiratory tract compared to human PIV3. Although the bovine strains were attenuated in these primates, the two viruses grew well enough to induce a neutralizing antibody response that, based upon previous observations, should provide resistance to human PIV3 infection. These encouraging observations provide a rationale for clinical evaluation of bovine PIV3 as a live vaccine for prevention of human PIV3 disease (Coelingh, Murphy).

Influenza A Virus, Avian-Human Influenza A Virus Reassortants. Previously it was demonstrated that avian influenza A viruses that are restricted in replication in the lower respiratory tract of monkeys can serve as donors of attenuating genes to human wild type influenza A viruses. Avian-human (AH) influenza A reassortant viruses that derived their hemagglutinin (HA) and neuraminidase (NA) genes from their wild type human influenza A virus parent and their six other ("internal") genes from their avian influenza A virus parent were regularly attenuated when tested in monkeys and human volunteers. These reassortant viruses replicate efficiently in tissue culture at 42°C, like their avian influenza A virus parent, and are restricted in replication in the respiratory tract of monkeys and volunteers (Snyder, Murphy).

Recent studies of a new avian influenza A virus parent (A/Pintail/Alberta/119/79) and its six-gene avian-human influenza reassortants indicated that these viruses were also restricted in replication in the respiratory tract of squirrel monkeys and were attenuated in humans. Analysis of reassortant viruses that derived one or more internal genes from the avian influenza A/Pintail/79 virus, while all other genes were derived from the human influenza A/Washington/897/80 (H3N2) virus, revealed an interesting new phenomenon, namely that a specific constellation of avian virus and human virus polymerase genes specified restriction of viral replication in the upper and lower respiratory tract of squirrel monkeys. This observation suggests a new vaccine strategy for construction of attenuated influenza A viruses. Thus, a reassortant virus containing the specific constellation of polymerase genes that restricts viral replication in primates could be used as a donor of this gene constellation to effect attenuation of influenza A virus antigenic variants as they emerge in nature (Snyder, Murphy).

Analysis of single avian influenza A virus gene reassortants also provided evidence that the M gene of the A/Pintail/79 virus did not play a role in attenuation of AH A/Pintail/79 reassortants. In contrast, previous studies indicated that the M gene of the A/Mallard/NY/6750/78 (H2N2) virus specified restriction of virus replication in the lower respiratory tract. Because the single avian M gene AH reassortants derived from the two avian influenza A virus donors were produced on a different background of human influenza A virus genes, it was necessary to isolate new single avian influenza M gene reassortants on the same background of human influenza A virus genes. This was done to exclude an effect of human influenza A virus genes on attenuation specified by an avian influenza A virus M gene. A reassortant virus which derived its M gene from the avian influenza A/Pintail/79 virus and all other genes from the human influenza A/Udorn/72 (H3N2) virus was isolated and its replication in the upper and lower respiratory tract of monkeys was compared to that of a comparable reassortant that derived its M gene from the avian influenza A/Mallard/78 virus and all other genes from the same human influenza A virus. The A/Mallard/78 reassortant virus was restricted in replication, while the A/Pintail/79 reassortant virus replicated as efficiently as the human wild type influenza A virus parent. This established unambiguously that the M genes of the two avian influenza A donor viruses differ in their ability to attenuate human influenza A viruses (Snyder, Murphy).

Analysis of the nucleotide sequence of the avian influenza A/Pintail/79 virus M gene and comparison of its deduced amino acid sequence with that of the avian influenza A/Mallard/NY/78 virus M gene, which does specify attenuation, revealed only four amino acid changes, three of which are conservative. None of these amino acid changes occurred at the previously defined sites of host species specific amino acid substitution. These observations suggest that only a single amino acid change may be responsible for the attenuation specified by the avian influenza A/Mallard/NY/78 virus M gene (Snyder, Murphy).

Influenza A Virus: Genetic Basis for Attenuation of Candidate Vaccine Reassortant Viruses Derived From Cold Adapted (ca) Donor Virus. In order to identify the genes of the cold adapted (*ca*) influenza A/Ann Arbor/6/60 (H2N2) donor virus that specify the *ca*, temperature sensitivity (*ts*), and attenuation (*att*) phenotypes, a set of six single *ca* gene substitution reassortant viruses were produced. Each of these reassortants contained a different single "internal gene" (i.e., a gene which encodes a protein other than the surface glycoproteins) derived from the *ca* donor virus, while all other genes were derived from the human influenza A/Korea/1/82 (H3N2) wild type virus parent. These single influenza *ca* gene substitution reassortant viruses were analyzed *in vitro* and in ferrets, hamsters, and seronegative adult volunteers. *In vitro* studies revealed that the PA gene specifies the *ca* phenotype. The PB2 gene plays a major role in specifying the *ts* phenotype, while the PB1 gene plays a lesser role. In ferrets it was observed that the PB1, PB2, PA and M genes each specified restriction of viral replication in the lower respiratory tract. Because the normal body temperature of the ferret is 39°C, it was possible that a virus with the *ts* phenotype might be more restricted in replication in ferrets than in an animal with a body termperature of 37°C. Therefore, the reassortant viruses were evaluated in hamsters which have a normal body temperature of 37°C, the same as that of humans. The PB2, PB1 and M genes also restricted viral replication in the lower respiratory tract of hamsters. Studies in seronegative adult volunteers indicated that the PA and M genes each contributed to restriction of viral replication in the upper respiratory tract. Taken together our data suggests that four genes: PB1, PB2, PA, and M independently contribute to attenuation of *ca* reassortant virus vaccines in humans. These observations provide an explanation for the observed stability of the attenuation phenotype of *ca* influenza A reassortant virus vaccines in humans (Snyder, Murphy).

Influenza A Virus: Comparison of AH and ca Influenza A Virus Reassortants in Volunteers. Previously it was observed that live influenza A reassortant viruses which derive their genes coding for surface glycoproteins from a wild type human influenza A H3N2 or H1N1 virus and their six other genes from either the avian influenza A/Mallard/NY/6750/78 (H2N2) virus or human influenza A/Ann Arbor/6/60 (H2N2) *ca* virus were safe, attenuated, and immunogenic in humans. Efficacy of *ca* vaccines was demonstrated in several clinical trials, and the single AH reassortant studied was also found to induce protective immunity. Although the response of seronegative adult volunteers to immunization with AH and *ca* vaccines appeared similar, the vaccines were evaluated in separate trials and thus it was not possible to determine whether the small differences observed between AH and *ca* vaccines reflected different properties of the two attenuated virus donors or simply differences among the wild type donor strains or among the volunteer populations tested. In order to address these questions directly, a side-by-side comparison of the infectivity and efficacy of *ca* and AH

A/Bethesda/1/85 (H3N2) and A/Texas/1/85 (H1N1) reassortant vaccines was performed. The AH and *ca* reassortant viruses were observed to be similar with regard to their level of attenuation, immunogenicity, infectivity, and efficacy. Studies have been initiated to evaluate systematically the response of seronegative children to AH and *ca* reassortant H1N1 and H3N2 virus vaccines (Snyder, Murphy).

Influenza A Virus: Instability of Phenotype Specified By a 36 Nucleotide In-Phase Deletion in The NS Gene. Dr. Maassab (U. of Mich.) recovered a spontaneous viable mutant of influenza A virus which had sustained an in phase 36 base deletion in the center of the NS1 protein coding region of the 8th gene. A reassortant virus bearing this mutant NS gene on a background of 7 wild type influenza A virus genes was isolated in LID and it was shown that the mutant gene specified the *ts* phenotype. Because of the relatively large size of the deletion in the mutant gene we were surprised to recover ts^+ virus from experimentally infected hamsters and chimpanzees. The phenotypically revertant ts^+ isolates possessed a truncated NS gene which was the same size as the mutant NS gene which had sustained the 36 base deletion. We are now analyzing the genetic changes responsible for loss of the *ts* phenotype after replication *in vivo*, but clearly an intragenic or extragenic suppression mutation occurred that corrected the *ts* phenotype specified by the 36 base deletion. The observation that the phenotype specified by a relatively large deletion mutation may not be stable *in vivo* is not only surprising but disconcerting because it indicates that large deletion mutations may not necessarily provide a stable attenuation phenotype for live virus vaccines (Snyder, Murphy, Chanock).

Rotaviruses

Immunoprophylaxis with Live Attenuated Viral Vaccine. Rotaviruses have emerged as the single most important etiologic agents of severe diarrhea of infants and young children in developed and developing countries. In long term cross-sectional studies of infants and young children hospitalized with diarrheal illnesses in developed countries, rotaviruses are associated with one-third to one-half of such admissions. Also, the importance of rotaviruses as a cause of severe diarrhea in developing countries has been demonstrated consistently. In a one year study of patients coming to a treatment center for severe diarrheal illness in Bangladesh, Black *et al.* found that rotaviruses were the pathogens most frequently detected in children less than 2 years of age; 46% of such illnesses were rotavirus positive. Thus, the need for an effective rotavirus vaccine is now clear. The goal of such a vaccine is to prevent severe rotavirus diarrhea during the first 2 years of life when this disease is most serious.

An animal rotavirus strain, rhesus rotavirus (RRV), is under intensive study in LID as a vaccine candidate. This simian rotavirus has not been recovered under natural conditions from man and is thus not a virus of the human heritage. Although the genes of RRV exhibit significant divergence in sequence from the corresponding genes of human rotaviruses, this simian rotavirus shares neutralization epitopes on the viral outer capsid protein VP7 with human rotavirus type 3 and is thus similar to viruses of this serotype when tested by neutralization. This Jennerian approach to immunization in which a vaccine strain is derived from a non-human host is now undergoing extensive clinical evaluation for safety and efficacy in various studies in the United States and abroad. Two field studies with the RRV vaccine have now been completed, 4 others are undergoing analysis or are in progress and one is about to begin. Most promising are the results from a clinical trial in 247 infants and young children in Venezuela in which vaccine efficacy was 93% for infants who were 1-5 months of age at the time of vaccination and 100% for the most severe form of rotavirus diarrhea. In this study, the predominant infecting strain belonged to the same serotype as the vaccine virus. However, the vaccine proved to be ineffective in two other studies in which the predominant infecting strain belonged to a different serotype than RRV. Thus, it appears that serotype specific immunity may be necessary to achieve satisfactory protection against rotavirus diarrhea. In response to this need we have pursued the development of a multivalent vaccine which would induce resistance to each of the human rotavirus serotypes. For this purpose RRV has been employed as a donor of attenuating genes that are transferred to reassortant rotaviruses which bear the major neutralization protein (VP7) of serotype 1, 2 or 4. These single gene substitution reassortants, which contain 10 RRV genes and the gene for a human rotavirus VP7 specifying serotype 1, 2 or 4, are now being evaluated in phase I clinical trials. To date these reassortants appear to be as attenuated and immunogenic as RRV (Kapikian, Flores, Midthun, Chanock).

Rotavirus Reinfection. The development of an effective strategy for immunization against rotavirus gastroenteritis requires that we understand the extent and duration of immunity to virus infection and disease. Since there are four rotavirus serotypes that are important in causing human disease, as well as 2 newly identified serotypes of yet undetermined importance, both homotypic and heterotypic immunity must be examined. In order to obtain more information about the extent and duration of homotypic and heterotypic immunity, a study of children who experienced more than 1 episode of rotavirus diarrhea has been initiated. The rotavirus strains in 6 paired stool specimens from Egyptian children who have had 2 episodes of rotavirus gastroenteritis have been successfully serotyped by ELISA. In 3 cases, the same serotype caused reinfection; in the other 3 cases, a different serotype caused reinfection. Clinical and epidemiological information concerning these infections and reinfections are being obtained in order to interpret the significance of our observations (Midthun, Kapikian).

Stability of the Rotavirus Genome. Rotaviruses appear to undergo genetic variation by genetic drift or genetic shift (gene reassortment). Previous descriptions of genomic heterogeneity of rotavirus isolates suggested that the rotavirus genome was subject to frequent mutation. This interpretation may not be correct because we recently observed that the genome of a rotavirus which persisted in a newborn nursery for 4 years or more was remarkably stable. This rotavirus strain persisted in the nursery over this long interval because it caused asymptomatic infection. In order to determine with greater precision if mutations occurred which were not detected by our previous RNA-RNA hybridization analysis, we sequenced the VP7 gene of 18 strains recovered at different times from the same nursery over a four year period. This analysis included the complete protein coding region of 5 such strains. The VP7 gene was selected for analysis because it is under the greatest immunologic pressure and hence, selection for mutations in this gene should be strongest. In addition, 11 different strains from the same nursery were analyzed for stability of the fourth gene which encodes VP3. Very few changes in sequence were detected in the strains examined. Overall, changes were detected in the VP7 and VP3 genes in positions which did not alter their amino acid sequence. This finding supports the view that the rotavirus genome does not undergo genetic drift with high frequency (Flores, Green).

Outer Capsid Proteins VP3 and VP7 Are Important, Independent Protective Antigens. Rotaviruses possess 2 outer capsid proteins, VP3 and VP7, which are independent neutralization antigens. This was established previously in LID during studies in which the VP3 and VP7 genes were segregated by gene reassortment and their products analyzed for capacity to induce neutralizing antibodies and to serve as a target for virus neutralization. During the past year these outer capsid proteins were analyzed for their capacity to induce immunity to infection and disease. For this purpose a single VP7 gene substitution reassortant was produced by mating porcine rotaviruses OSU (serotype 3) and Gottfried (serotype 4). The resulting reassortant possessed the VP3 gene of one parent and the VP7 gene of the other parent. Newborn, colostrum deprived piglets (which lacked maternally-derived antibodies) were infected with OSU, Gottfried or the reassortant. The piglets developed neutralizing antibodies directed against both VP3 and VP7 and the titer of these two types of antibodies was similar. Three weeks later the piglets were challenged with OSU or Gottfried. Piglets infected at birth with the reassortant exhibited significant resistance to challenge with either parental virus indicating that VP3 and VP7 each play an important, independent role in host immunity to rotavirus infection and disease. The observation that a reassortant rotavirus bearing the VP3 of one serotype and the VP7 of another serotype induced resistance to both serotypes suggests an alternate strategy for immunization against human rotavirus disease (Hoshino, Kapikian).

Sequence Analysis of the Fourth Gene Product, VP3, and Role of This Outer Capsid Protein in Virulence. Previously when the viral RNAs of rotavirus strains (representing all 4 serotypes) which had been isolated from asymptomatic newborn infants ("nursery strains") were compared by RNA-RNA hybridization with strains from ill infants (also representing all 4 serotypes), a marked difference in the sequence of the fourth gene was detected between the two groups of rotaviruses. The fourth gene was observed to be highly conserved among nursery strains, but it differed significantly from the corresponding conserved gene of strains isolated from ill infants. The conserved fourth gene of the nursery strains probably plays a role in their attenuation because none of the other 10 rotavirus genes were conserved among these asymptomatic viruses. The molecular epidemiology of this phenomenon was further analyzed using a dot/blot hybridization procedure which discriminates the fourth gene of virulent and asymptomatic strains. Additional rotavirus strains recovered from a hospital newborn nursery in which nosocomial transmission of asymptomatic rotavirus infection has persisted for 4 years or more were analyzed. Each of the 11 strains studied possessed the conserved fourth gene associated with asymptomatic infection of newborns. Also, only one of 110 human rotavirus strains recovered from ill infants or children possessed the conserved fourth gene of the asymptomatic newborn strains. However, several strains recovered from sporadic asymptomatic newborn infection possessed a "conserved virulent" fourth gene. It should be noted that these infections did not occur in a nursery in which persistent transmission of asymptomatic infection had been documented. Hence, the association of a conserved fourth rotavirus gene sequence with persistent transmission of asymptomatic infection in the newborn nursery still holds. The sporadic occurrence of asymptomatic rotavirus infection in the newborn caused by a strain possessing the conserved fourth gene sequence of virulent strains suggests that host factors also play an important role in expression of virulence (Flores, Green).

Conservation of VP3 sequence among asymptomatic newborn strains of rotavirus and the conservation of a different set of sequences among virulent viruses was confirmed last year by sequence analysis of the N-terminal one third of the fourth gene of 4 asymptomatic strains (representing the 4 human rotavirus serotypes) and 4 virulent strains (also representing the 4 human serotypes). This year our analysis was extended to include the remaining two thirds of the fourth gene. RNA segment 4, which encodes VP3, is 2359 base pairs in length and contains a single long open reading frame of 2325 bases capable of coding for a protein of 775 amino acids flanked by 5' and 3' noncoding regions of 9 and 25 nucleotides, respectively. Alignment of amino acid sequences of the VP3 protein of human rotavirus strains indicates a high degree of homology (96% or more) among the asymptomatic viruses (serotypes 1, 2, 3 and 4), while homology between asymptomatic strains and virulent viruses is considerably less (76%). A high degree of conservation of VP3 amino acid sequence (93%) is also observed among 3 of the virulent strains (serotypes 1, 3 and 4). The VP3 of the remaining virulent strain (serotype 2) is more closely related to that of the other virulent viruses than to the VP3 of the asymptomatic strains. Eighty-six amino acid positions on VP3 outside the cleavage region were conserved among asymptomatic rotaviruses, while a different amino acid was conserved at each of these sites among virulent rotaviruses. At this time it is not possible to determine the relative contribution to virulence of the various sequences of the fourth gene product which are unique to asymptomatic or virulent rotavirus strains (Gorziglia, Green, Flores, Nishikawa, Taniguchi, Chanock).

The deduced amino acid sequence of the VP3 protein of 4 virulent and 4 asymptomatic rotavirus strains indicates that 16 regions (7 to 64 amino acids in length) are completely or almost completely conserved among both asymptomatic and virulent rotaviruses. These conserved regions probably play a critical role in infectivity and other structural activities of rotavirus. There are 14 variable regions (6 to 70 amino acids in length) among the 8 human virulent and asymptomatic rotaviruses which were analyzed. It is likely that some of the variable regions contain neutralization epitopes. Significantly, there are 7 regions (9 to 38 amino acids in length) conserved among the 4 virulent human rotaviruses, while a different set of sequences are conserved among the asymptomatic human rotaviruses. These sequences may also play a role in virulence (Gorziglia, Green, Flores, Nishikawa, Taniguchi, Chanock).

VP3 Neutralization Epitopes. Neutralization epitopes were identified on VP3 by sequence analysis of antigenic mutants of simian rotavirus SA-11 which were no longer neutralized by the polyvalent hyperimmune antiserum or neutralizing monoclonal antibody (N-mAb) used for their selection. These analyses identified amino acid substitutions in 8 separate regions of VP3. Variable region 8 (amino acids 386-397) appears to contain an immunodominant neutralization epitope because each of the 7 "neutralization escape" mutants sustained an amino substitution in position 393. It is of interest that escape mutants of a human serotype 1 virus selected with a cross-reactive VP3 N-mAb also sustained an amino acid substitution at position 392 (which corresponds to residue 393 of SA-11). Variable region 4 (amino acids 133-149) probably also contains a neutralization epitope (Taniguchi, Gorziglia, Nishikawa).

VP7 Neutralization Epitopes. Antigenic mutants of SA-11 rotavirus were selected by Dr. Ward (Cincinnati) during 39 passages in culture in the presence of SA-11 hyperimmune antiserum. The VP7 gene of these mutants, which were no longer neutralized by the antiserum used for their selection, was sequenced in LID to determine the position and total repertoire of neutralization epitopes present on the two major surface proteins which constitute the protective antigens of simian rotavirus SA-11. For this purpose we assumed that sites of amino acid substitution corresponded to actual epitopes. Previously, Dyall-Smith (Australia) analyzed SA-11 mutants selected with N-mAbs which represented 4 different antigenic specificities on the VP7 outer capsid protein. His analysis identified VP7 neutralization epitopes in variable regions V2 (amino acid residue 96), V4 (residue 147) and V5 (residues 211 and 223). Our sequence analysis of 7 SA-11 mutants originally selected with SA-11 hyperimmune antiserum also identified amino acid substitutions in these three variable regions of VP7. In addition to these amino acid changes, we found that variable region V1 (residues 69-73), which corresponds to the unique glycosylation site of the SA-11 VP7, was also the site for an amino acid substitution in each of the 7 antigenic mutants. Thus, this region probably represents another important and heretofore unrecognized neutralization epitope on VP7. Also, variable region V6 (residue 238) could be a potential neutralization epitope, because amino acid substitutions were observed in this region in 4 of the 7 mutants. Sequence analysis of antigenic mutants of serotype 1 or serotype 4 rotaviruses selected with the appropriate serotype-specific N-mAbs indicated that amino acid substitutions occurred at sites that corresponded exactly to putative epitopes in variable regions V2, V4 and V5 of simian SA-11 virus. Comparison of amino acid sequences of VP7 from a variety of strains indicate that these regions are conserved for strains within a given serotype, but exhibit considerable divergence among strains belonging to other serotypes. Thus, epitopes responsible for serotype specificity are located in the same sites on the linear sequence of VP7 irrespective of sequence divergence at these sites among viruses of different serotype. Most neutralization epitopes appeared to be independent of each other because amino acid substitution in one site did not generally affect the binding of specific N-mAbs to other sites. However, in one instance epitopes appeared to be interrelated because amino acid substitution in one site affected binding of N-mAbs to another distant site (Gorziglia, Taniguchi, Nishikawa).

Sequence Diversity of VP7. Antigenic differences among rotaviruses detected by neutralization were investigated at the amino acid level by nucleotide sequence analysis of the gene coding for the major serotype-specific neutralization protein (VP7) of human rotavirus strains D, Montgomery, M37, DS1, HN126, Price, ST3, and VA70. The deduced VP7 amino acid sequences of these viruses were compared and six regions of sequence-divergence were identified on the VP7 of strains belonging to 4 different serotypes. These six regions of amino acid divergence were highly conserved within viruses of the same serotype. It is unlikely that only one or a few amino acids determine the serotype specificity of a rotavirus, instead a series of consensus sequences appear to specify each serotype. Two such divergent regions of the VP7 gene were analyzed for twenty rotavirus field isolates of unknown serotype. Each of the 20 strains analyzed exhibited marked homology in these 2 regions to only one of the 4 human rotavirus serotypes. The serotype predicted by sequence analysis correlated well with the serotype subsequently identified by ELISA. Thus, it is possible to sequence the VP7 gene of a viral isolate and predict its serotype based upon homology to a consensus sequence of a known serotype. Although this would not be a practical method for most laboratories, it may prove useful in characterizing strains which cannot be identified by neutralization or monoclonal antibody ELISA (Green, Flores).

Two new human rotavirus serotypes were recently recognized. The VP7 gene of both strains was sequenced and comparison of these sequences with those of the 4 established human rotavirus serotypes revealed significant divergence in the variable regions. Thus, sequence analyses confirmed that the 2 strains represent new serotypes (Green, Hoshino, Flores).

Sequence Analysis of the Major Inner Capsid Protein VP6 Which Bears Subgroup Antigens. The nucleotide sequence of the sixth gene that codes for the major inner capsid protein, VP6, of human rotavirus 1076 (subgroup I), porcine rotavirus Gottfried (subgroup II), equine rotavirus H-2 (neither subgroup I nor II) and equine rotavirus FI-14 (both subgroup I and II) was determined. The sixth segment (+) strand RNA has a 5' and 3' nontranslated sequence of 23 and 142 nucleotides respectively, and encodes a VP6 protein of 399 amino acids. Alignment of amino acid sequences of the VP6 protein of strain FI-14 and subgroup II rotaviruses (Wa and Gottfried) indicated a high degree of homology (94%), while homology between strain FI-14 and subgroup I rotaviruses (SA-11, RF, and 1076) was somewhat less (90-92%). On the other hand, a high degree of conservation of amino acid sequence (95-97%) was observed between the H-2 strain and subgroup I rotaviruses. Five regions that may contribute to subgroup epitopes were identified. Region A (amino acids 45, 56) and region C (amino acids 114, 120) may contribute to subgroup I epitopes and regions B (amino acids 83, 86, 89, 92), D (amino acids 312 or 314, 317 or 319) and E (amino acids 341 or 343, 350 or 352) may contribute to subgroup II epitopes. Monoclonal antibodies directed at VP6 epitopes shared by all rotaviruses reacted by Western blot with both monomeric and trimeric forms of VP6, while monoclonal antibodies specific for a subgroup I or a subgroup II epitope reacted only with the trimeric form of VP6. This observation and the sequence analyses suggest that subgroup antigenic specificity is determined by conformational epitopes produced by the folding of VP6 or the interaction between VP6 monomers (Gorziglia, Hoshino).

Serotype Identification by Hybridization With Labeled Rotavirus Probes. The rotavirus VP7 neutralization antigens of serotypes 1, 2, 3 and 4 were analyzed for genetic relatedness by hybridization of their VP7 genes using single human rotavirus gene substitution reassortants. Double-stranded (ds) genomic RNAs extracted from tissue culture adapted human rotaviruses (serotypes 1-4) were hybridized to single-stranded (ss) mRNA probes derived from each of the human-bovine rotavirus reassortants with the antigenic specificity of serotype 1, 2, 3 or 4. These reassortants derived only their VP7 gene from their human rotavirus parent. When stringent conditions were used, bovine rotavirus genes did not hybridize to the corresponding genes of human rotaviruses and thus, hybridization occurred only between the human rotavirus VP7 gene in the labeled probe and a related VP7 gene present in the human rotavirus under study. A high degree of homology was demonstrated between the probe and the VP7 gene of strains belonging to the same serotype, whereas hybrid bands did not form with VP7 genes from human rotaviruses belonging to a different serotype. It appears that serotype identification by hybridization with the reassortant probes is both sensitive and specific and circumvents the need for tissue culture adaptation of human rotaviruses since the genomic RNAs are purified directly from stool specimens (Midthun).

Dengue Virus

Dengue viruses (types 1 through 4) continue to pose a major public health problem in many geographic areas, especially in Southeast Asia, the South Pacific, the Caribbean and in Central and South America. In addition to causing the classical "break bone fever" syndrome, dengue viruses can also cause a hemorrhagic fever/shock syndrome which has a high mortality rate in children and young adults. In regions of the world where it occurs, dengue hemorrhagic fever-shock syndrome constitutes a leading cause of death in young children. Unfortunately, there are no specific immunoprophylactic measures available against dengue and prevention of dengue infection still depends upon mosquito vector control which is labor-intensive and often ineffective. Hence, WHO has assigned a high priority to the development of an effective dengue virus vaccine.

Like other members of the flavivirus family, extracellular dengue virus has a relatively simple structure. Virions contain only three viral-coded proteins, designated capsid protein (C), membrane protein (M) and surface envelope (E) glycoprotein. On the other hand, intracellular virus, which is also infectious, lacks M but instead contains another glycoprotein (PreM) from which M is derived by cleavage. Both C and M are internal proteins. The E glycoprotein is responsible for adsorption of virus to the cell surface and appears to the major target for neutralization of infectivity by specific antibodies. In addition, several nonstructural proteins have also been identified in dengue virus-infected cells. Among them is NS_1, a glycoprotein which was originally described as the soluble complement fixing antigen. NS_1, which is displayed on the surface of infected cells, may also play an important role in mediating immunity since the analogous NS_1 glycoprotein of yellow fever virus has been shown to be a protective antigen in mice and in primates. Recently the NS_1 of dengue type 2 virus was also shown to be a protective antigen.

Sequence Analysis of Dengue Type 4 Viral Genome. The single strand viral RNA genome of dengue type 4 virus (strain 814669) has been completely sequenced. The genome contains 10,644 nucleotides with a single long open reading frame that encodes a polyprotein of 3386 amino acids. The polyprotein is cleaved post-translationally into the full complement of viral proteins by a mechanism that probably is similar to that proposed for gene expression of other flaviviruses. Homology alignment of the dengue type 4 virus polyprotein with the polyproteins of two other flaviviruses, yellow fever and West Nile viruses, indicated that the 5' end of the dengue viral RNA encodes the three structural proteins designated C, M and E. The 3' terminal 8200 nucleotides encode seven nonstructural proteins designated NS_1, NS_{2A}, NS_{2B}, NS_3, NS_{4A}, NS_{4B}, and NS_5. NS_1 is the major NS glycoprotein produced during viral infection, while NS_3 and NS_5 contain polymerase-like amino acid sequences. Except for NS_1, the significance of dengue NS proteins is not known (Zhao, Lai).

Expression of Dengue Viral Proteins by a Vaccinia Virus Recombinant. Following sequence analysis of the complete dengue viral genome and construction of a full length cDNA clone of the genome, we sought to express both protective antigens; i.e., the E and NS_1 glycoproteins, from cloned dengue cDNA using vaccinia virus as a vector. A recombinant vaccinia virus containing cloned DNA sequences (4.1Kb Bgl II fragment) coding for the three structural proteins and nonstructural proteins NS_1 and NS_{2a} of dengue type 4 virus was constructed. The dengue sequences were placed under the control of the vaccinia P7.5 early-late promoter. CV-1 cells infected with this recombinant virus produced dengue structural proteins as well as the nonstructural protein NS_1. Proteins precipitated from lysates of recombinant virus infected cells by specific antiserum were similar to authentic viral proteins with respect to size and glycosylation pattern.

The recombinant vaccinia virus (10^8 pfu) was used to immunize cotton rats by the intradermal route and their immune response was evaluated by serologic analysis. Immunized animals did not develop dengue specific antibodies measurable by ELISA, plaque reduction virus neutralization, or hemagglutination-inhibition. However, all the cotton rats developed a high titer of serum neutralizing antibodies against vaccinia virus (1:1000 to 1:5000) indicating that they had been infected with the recombinant virus. Radio-immunoprecipitation of ^{35}S-labeled dengue virus proteins was also attempted in an effort to detect low levels of dengue-specific antibodies. NS_1 glycoprotein was precipitated by the serum of one animal obtained six weeks after immunization. However, the same antiserum did not precipitate either the E or the Pre M glycoprotein. Sera from ten other animals were completely negative by this assay. Thus, the recombinant vaccinia virus failed to elicit a detectable seroresponse to the E or the Pre M glycoprotein. There are several factors which may have contributed to the poor immune response to the NS_1 glycoprotein as well as our failure to detect a seroresponse to the E glycoprotein. Most important was the low level of expression of dengue structural proteins and NS_1 by the vaccinia virus recombinant. The amount of dengue viral proteins produced in cells infected with the recombinant virus was approximately one tenth that observed in cells infected with dengue type 4 virus. Translation of the dengue chimeric gene transcript from the recombinant virus may not have been optimal because a region of the 5' noncoding sequence of dengue, not included in the recombinant, might be required for efficient translation of the dengue polyprotein. Also, expression of dengue proteins by the vaccinia recombinant may have been reduced because optimal processing of the polyprotein may require participation of proteins produced by the downstream nonstructural genes not included in the recombinant. These possibilities can now be explored by constructing vaccinia recombinants which include more of the 5' noncoding region of dengue virus or the downstream 3' sequences. Also, other vaccinia promoters will be examined in an attempt to increase the level of expression of dengue structural proteins and NS_1. In addition, poor immunogenicity could result in part from inherent properties of the 2 protective glycoproteins because during viral infection the E glycoprotein accumulates only intracellularly and the NS_1 nonstructural glycoprotein is not expressed abundantly on the cell surface (Zhao, Lai, Chanock).

Expression of Dengue Viral Proteins by a Baculovirus Recombinant. The baculovirus expression system was also used for the synthesis of dengue viral proteins from cloned DNA sequences. A stable baculovirus-dengue recombinant was constructed which contained the 4.1 Kb Bgl II dengue DNA fragment coding for the three structural proteins [capsid (C), matrix (M) and envelope (E)] and nonstructural proteins NS_1 and NS_{2A}. Dengue Pre M, E and NS_1 glycoproteins were expressed at a high level by the baculovirus recombinant during infection of insect sf9 cells as indicated by intense staining of infected cells by fluorescein labeled antibodies specific for each of these antigens. This indicated that the dengue DNA sequence was transcribed and the resulting mRNA was properly translated in insect cells to produce these dengue glycoprotein products. Analysis of proteins produced in baculovirus recombinant infected cells by SDS-polyacrylamide gel electrophoresis followed by "western" blotting allowed us to identify E, NS_1 and Pre M which were similar in size to authentic proteins produced during dengue virus infection. These observations suggested that proteolytic cleavage and glycosylation of these dengue proteins was normal in the baculovirus expression system. Immunization of rabbits with lysates from baculovirus recombinant infected sf9 cells induced antibodies specific to each of the three dengue glycoproteins as indicated by radio-immune precipitation. However, these antibodies did not neutralize viral infectivity or inhibit viral hemagglutination, probably because their titer was too low. Nonetheless, a preliminary immunization-challenge study performed in mice suggests that dengue proteins expressed by the baculovirus recombinant are both immunogenic and capable of inducing resistance to dengue virus disease. In a collaborative study with Dr. K. Eckels (WRAIR) it appeared that immunization of mice with the lysate of 5×10^7 baculovirus recombinant-infected sf9 cells induced resistance to fatal encephalitis produced by intracerebral inoculation of dengue type 4 virus (Zhang, Lai).

Attempts to Increase Immunogenicity of Dengue Viral Glycoproteins by Expression at the Cell Surface. In order to improve the immunogenicity of dengue glycoproteins produced by recombinant viruses we sought to modify the viral vector so that these dengue glycoproteins were expressed on the cell surface or secreted extracellularly. In previous studies vaccinia virus recombinants which expressed viral glycoproteins on the cell surface successfully induced protective immunity. The vaccinia-RSV G glycoprotein recombinant is an example of such a successful recombinant. This RSV glycoprotein was shown to anchor itself in the membrane of infected cells by means of a hydrophobic sequence of amino acids near its N-terminus. Advantage was taken of this property of the RSV G glycoprotein to engineer chimeric proteins consisting of an N-terminal portion of RSV G fused in-frame to a large fragment (80%) of dengue E glycoprotein or to smaller subfragments of E. It was hoped that the RSV portion of such chimeras would provide the sequence required for processing and transport to the cell surface. Three different truncated forms of the RSV G gene had previously been constructed by Dr. N. Elango, LVD, using the technique of site-specific mutagenesis. These three truncated sequences code for the N-terminal 70, 179 and 229 amino acids of G, respectively.

Recombinant DNA techniques were employed to construct chimeric genes encoding a truncated portion of the RSV G glycoprotein ligated in-frame to 80% of the dengue type 4 envelope (E) glycoprotein gene (Hae III fragment) or to a smaller portion of the E gene. These genes were inserted into vaccinia virus, under control of the P7.5 promoter, to obtain expression of the chimeric proteins in infected cells. Initially, truncated RSV G sequences were ligated to a fragment of the dengue E gene coding for 380 amino acids which represent about 80% of the length of this glycoprotein. Chimeric proteins expressed by vaccinia virus were recognized by hyperimmune dengue virus antiserum and by monoclonal antibodies to E, but cell-surface localization could not be demonstrated, and immunization of cotton rats failed to induce an antibody response to E. Recombinants coding for subfragments of E, ranging from 28 to 70% of the protein, have been constructed and will be tested in the same manner. This approach may aid in localizing epitopes recognized by monoclonal antibodies, and if immunization using subfragments is successful, it will be possible to determine antigenic sites which induce a neutralizing antibody response (Bray, Markoff, Lai).

In another approach to cell surface expression of dengue viral glycoproteins the hemagglutinin (HA) of influenza A virus was chosen to provide the N-terminal signal peptide as well as the C-terminal anchor sequences that are required for intracellular protein transport and cell surface expression and anchorage. DNA sequences coding for the E glycoprotein were constructed by joining the Sal I/Acc I cleaved dengue DNA fragment to chemically synthesized oligomers. In order to prepare HA as an expression vector, internal DNA sequences of HA were removed by XbaI and XhoI digestion and deletion was extended from the cleavage sites by digestion with Bal 31. This collection of HA deletions will be used for insertion of the E DNA sequences and expression of the recombinant DNA in an SV40 expression vector. Recombinant SV40 virus producing a chimeric HA-E protein which is displayed on the cell surface or secreted extracellularly will be identified by immunofluorescence assay. Finally, the DNA sequences expressing the chimeric protein will be isolated and used for construction of a recombinant vaccinia virus (Lai, Bray).

Also studies are underway to determine whether the large (Hae III) fragment of E contains sequences which act to prevent it from traversing the Golgi and reaching the plasma membrane. Thus, attempts have begun to express the Hae III fragment of E downstream from a cleaveable influenza A HA signal sequence in an SV40 expression vector, pSV2330. The SV40/HA signal/Hae III E recombinants will be scored for secretion of the E fragment coded for by the Hae III dengue genomic segment (Markoff).

Efforts are also underway to express the NS$_1$ glycoprotein at the cell surface in high concentration. Expression of this protective antigen at the surface of infected cells in a higher concentration than occurs during dengue virus infection would be particularly attractive as a strategy for immunization, because immunity induced to this nonstructural antigen would not be mediated by virus neutralization and hence the possibility of immune enhancement (i.e., antibody mediated enhancement of viral adsorption) could be avoided. Currently, recombinant vaccinia viruses are being constructed for expression of the entire NS$_1$, or various subsets of NS$_1$ fragments, on the cell surface. A DNA clone coding for almost the entire NS$_1$ as well as two DNA subclones have been produced. One subclone codes for the first 267 amino acids of NS$_1$ and the other encodes amino acids 267 to 383. Construction of recombinant vaccinia viruses containing these NS$_1$ sequences are in progress (Falgout, Lai).

Identification of Linear Epitopes on the Dengue Envelope and NS$_1$ Glycoproteins.

The antigenic structure of the envelope (E) glycoprotein of dengue virus was studied by analyzing the antigenicity of 38 overlapping "nested" peptides (synthesized by our collaborators at the Research Institute of the Scripps Clinic) which span the predicted amino acid sequence of the E glycoprotein of dengue virus type 4, strain 814669. The genome of this strain was previously cloned and sequenced in our laboratory. ELISA was used to detect antibody binding. Eight distinct linear epitopes were identified. Two peptides reacted strongly with polyclonal hyperimmune mouse ascitic fluid directed against strain 814669 (O.D. max.≧1.2; titer ≧1:1,000) and 4 peptides clustered at the NH$_2$-terminus of the molecule reacted weakly with the same ascitic fluid (O.D. max 0.5-0.6; titer ≦ 1:80). Two additional peptides were observed to bind 2 monoclonal antibodies (mabs) directed against a closely related dengue 4 strain (H241). One of these mabs neutralized strain H241. Currently, peptide antibodies are being prepared in rabbits. Preliminary results indicate that antiserum to one peptide (73B), that strongly binds polyclonal anti-814669 ascitic fluid, immune precipitates E and neutralizes strain 814669 at a titer of 1:10 in a plaque reduction assay. Protection studies in mice using peptide 73B as an immunogen and expression of the sequence coding for 73B at the surface of vector-infected cells are planned. In a related study of NS_1, the nonstructural glycoprotein of dengue virus which is thought to be the target for antibody mediated killing of infected cells, an additional 13 peptides were analyzed which were scattered throughout the linear sequence of the dengue type 4 virus NS_1. One of these peptides was strongly reactive with polyclonal hyperimmune 814669 mouse ascitic fluid in ELISA. Additional NS_1 peptides are being synthesized so that a complete overlapping set will soon be available to finish our analysis of this protective protein (Markoff, Bray, Chanock).

Attempts to Construct Infectious Dengue Virus cDNA. Genetic manipulation of the viral genome at the molecular level is an attractive approach to development of stable, live attenuated vaccine viruses for prevention of viral disease. Hence, attempts were undertaken to produce: (a) full-length cloned dengue DNA and (b) its RNA transcript synthesized in vitro which are infectious after introduction into permissive mosquito or primate cells. Initially, seven overlapping DNA segments spanning the entire dengue type 4 virus genome were used to construct a complete DNA copy by joining DNA fragments cleaved by endonucleases at shared sites. The unit-length dengue DNA of 10,644 nucleotides appeared to be stable in plasmid pBR322 as a single molecule as shown by restriction enzyme digestion. The following sequences were added to the dengue recombinant DNA in an attempt to increase infectivity of dengue nucleic acid: (1) an SV40 promoter to increase transcription of RNA from dengue DNA following its transfection into primate cells; (2) an SP-6 promoter to increase in vitro synthesis of RNA from a dengue DNA template. Under assay conditions which were efficient for detection of infectivity of dengue virion RNA, we failed to detect infectivity of either dengue DNA or its RNA transcripts in cell culture. Presence of a short length of non-dengue genomic sequences at the 5' and 3' ends of the complete dengue cDNA may be responsible for its lack of infectivity. Experiments are currently in progress to remove these additional non-viral nucleotides so that only precise dengue RNA sequences are transcribed in vitro or in cell culture, including a 7mG cap at the 5'-terminus (Zhao, Lai).

Honors and Awards

Robert M. Chanock

Member of Scientific Review Committee of Research Institute of Scripps Clinic, La Jolla, CA., Jan. 1987.

Invited to present the Second Annual Harry A. Feldman Memorial Lecture "Respiratory Syncytial Virus: What we have learned in the past 30 years", American Epidemiological Society (60th Annual Meeting), Medical University of S. Carolina, Charleston, S.C., March 1987.

Section and Class Meetings of National Academy of Sciences, April 1987.

Chairman of Virology Session of Scholars In Residence NIH Centennial Symposium "The Impact of Modern Biology on Health Research," NIH, May 1987.

Member of WHO Steering Committee on Acute Respiratory Viruses of World Health Organization (WHO) 1987.

Co-chairman of NIAID-WHO Workshop on Respiratory Syncytial and Parainfluenza Viruses, NIH, May 1987.

Invited speaker at Nobel Symposium on "The Vaccines of the Future", Karlskoga, Sweden, Aug. 1987.

Invited speaker at Karolinska Institut, Stockholm, Sweden, Aug. 1987. Topic: "Progress in Viral Vaccines".

Co-chairman of Fifth Conference on Modern Approaches to Vaccines, "Modern Approaches to New Vaccines Including Prevention of AIDS", Cold Spring Harbor Laboratory, Sept. 1987.

Member of the "AIDS Immunoglobulin Clinical Trial Working Group" of NIAID and NHLBI, 1987.

Albert Z. Kapikian

Invited to participate in Ciba Foundation Symposium No. 128, "Novel Diarrhea Viruses" in London, England, July 14-17, 1986.

Invited as WHO temporary adviser to attend the second meeting of the Scientific Working Group on Immunology, Microbiology and Vaccine Development of the Diarrheal Diseases Control Program of the World Health Organization. Geneva, Switzerland, October 7-10, 1986. Dr. Kapikian is a member of the Scientific Working Group.

Invited to be member (ad hoc) of review panel (Biotechnology/Immunology Panel [Bacterial and Viral Disease]) of Program in Science and Technology Cooperation of Agency for International Development, October 14, 1986, at National Academy of Scineces at Joseph Henry Building, Washington, D.C.

Invited member U.S. Panel of Japan-U.S. Cooperative Medical Science Program 1986.

Invited to make presentation at Theobold Smith Society of New Jersey Branch of American Society for Microbiology Workshop: Current Concepts in Diagnosis and Treatment of Urinary and Gastrointestinal Tract Infections, October 29, 1986, at Woodbridge Hilton, South Iselin, New Jersey. Presentation on "Viral Enteric Pathogens".

Co-chairman (with Dr. Konno) of 20th Joint Viral Panel Meeting of Japan-U.S. Cooperative Medical Science Program Working Conference on Viral Diseases in Nagasaki, Japan, November 18-20, 1986.

Presentation on "Further Studies on the 'Jennerian' Approach to Vaccination Against Infantile Diarrhea with a Rhesus Rotavirus Vaccine Candidate" at 20th Joint Viral Panel Meeting of the Japan-U.S. Cooperative Medical Science Program Working Conference on Viral Diseases in Nagasaki, Japan November 18-20, 1986. Presentation on November 19, 1986.

Presentation on "Evaluation of Rhesus Rotavirus x Human Rotavirus Reassortants with the Neutralization Specificity (VP7) of Human Rotavirus Type 1 or 2 as Vaccine Candidates in Humans" at 20th Joint Viral Panel Meeting of the Japan-U.S. Cooperative Medical Science Program Working Conference on Viral Diseases in Nagasaki, Japan, November 18-20, 1986. Presentation November 19, 1986.

Invited to make presentation at "Symposium on Progress in Vaccinology" December 1-5, 1986, New Delhi, India, at National Institute of Immunology. Presentation on "Rationale for the Development of a Rotavirus Vaccine for Infants and Young Children" on December 2, 1986.

Invited to make presentation at All India Institute of Medical Sciences. Presentation on "Recent Advances in Viral Gastroenteritis" on December 4, 1986. New Delhi, India.

Invited to be chairman of closing session of "Symposium on Progress in Vaccinology" at National Institute of Immunology on December 5, 1986 in New Delhi, India.

Invited as temporary adviser to 3rd meeting of the WHO Scientific Working Group on Immunology, Microbiology and Vaccine Development of the Diarrheal Diseases Control Programme of WHO on February 17-20, 1986 in Geneva, Switzerland. Dr. Kapikian is a member of this Scientific Working Group.

Invited to make presentation at Seminar "Virus Structure and Identification" at Annual Meeting of the American Society for Microbiology, Atlanta, Georgia. March 1-6, 1987. Presentation on "Use of Immunoelectron Microscopy as an Epidemiological Tool for Detection, Identification, and Characterization of Fastidious Viral Agents" on March 2, 1987.

Membership Committee, American Epidemiological Society.

Recipient of 1987 Behring Diagnostics Award in Diagnostic Virology and Rickettsiology of the American Society for Microbiology, in Atlanta, GA., March 1, 1987 at the ASM Annual Meeting.

Invited speaker, Modern Approaches to New Vaccines Including the Prevention of AIDS meeting, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, Sept. 9-13, 1987, "Development of a rotavirus (RV) vaccine by a "Jennerian" and a modified "Jennerian" approach".

Ching-Juh Lai

Invited symposium speaker, VIIth International Congress of Virology, Edmonton, Alberta, Canada, Aug. 9-14, 1987.

Brian R. Murphy

Consultant to U.S. Army Medical Research Institute of Infectious Diseases, "VEE Vaccines for Man", Fort Detrick, Md., Dec. 1986

Co-inventor, United States Patent (number pending) Temperature sensitive reassortant viruses and vaccine against equine influenza, Feb. 1987

Invited lecturer at Johns Hopkins School of Medicine, University of Maryland at College Park, and University of Maryland School of Medicine, Baltimore, MD. Mount Sinai Medical Center, 1987.

Consultant, World Health Organization

State-of-the-Art lecture at the American Society of Virology meeting, Chapel Hill, N.C., June 1987

Chairman, Vaccines II session, American Society of Virology meeting, Chapel Hill, N.C., June 1987

Robert H. Purcell

Invited speaker, IXth International Congress of Infectious and Parasitic Diseases, Munich, Ger., July 20-26, 1986.

Invited participant, WHO Viral Hepatitis Control Program, Munich, Ger., July 25-26, 1986.

Invited participant, NIAID AIDS Vaccine Workshop, Bethesda, MD, July 28-29, 1986.

Invited speaker, Annual Meeting of the American Association for the Study of Liver Diseases, Chicago, ILL, Nov. 2-5, 1986.

Coorganizer and participant, Workshop on Epidemic Non-A, Non-B Hepatitis, NIH, Bethesda, MD., Nov. 19-20, 1986.

Invited speaker, Symposium on Progress in Vaccinology, New Delhi, India, Dec. 1-5, 1986.

Invited speaker, Hepatitis Workshop, Palmas Del Mar, San Juan, Puerto Rico, Jan. 28-30, 1987.

Invited speaker, Symposia on Hepatitis B Virus and AIDS Viruses, Keystone, Colorado, Mar. 30-Apr. 4, 1987.

Invited speaker, The Hans Popper Lecture, Symposium on Current Concepts in Liver Pathology, Columbia University College of Physicians and Surgeons, New York City, N.Y., Apr. 30-May 1, 1987.

Member, Organizing Committee and Invited Speaker, International Symposium on Viral Hepatitis, London, Eng., May 25-28, 1987.

Invited participant and Member of Committee, Meeting of WHO Steering Committee on Hepatitis A and Polio Vaccines, London, May 29-30, 1987.

Judy Beeler

Invited speaker, American Society for Virology, 1987 annual meeting, Chapel Hill, NC, May 31-June 4, 1987, "Biological characterization of neutralizing monoclonal antibodies elicited by the fusion glycoprotein of respiratory syncytial virus".

Kathleen Coelingh

Invited speaker, St. Jude Children's Research Hospital, Division of Molecular Virology, "Antigenic and functional properties of type 3 parainfluenza virus HN and its expression by recombinant baculoviruses", Memphis, TN, May 1987.

Invited speaker, NIAID-WHO workshop on Respiratory Syncytial and Parainfluenza Type 3 Viruses, "Human and bovine type 3 parainfluenza viruses: antigenic structure and molecular epidemiology", May 4-5, 1987.

Recipient of World Health Organization grant for "Antigenic and molecular characterization of the glycoproteins of respiratory syncytial virus and antibody-resistant variants".

Invited speaker, Modern Approaches to New Vaccines Including the Prevention of AIDS meeting, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, Sept. 9-13, 1987, "Development of live virus and subunit vaccines for parainfluenza type 3 virus".

Jeffrey Cohen

Invited speaker, International Symposium on Viral Hepatitis, London, Eng., May 25-28, 1987.

Invited speaker, Modern Approaches to New Vaccines Including the Prevention of AIDS meeting, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, Sept. 9-13, 1987, "Use of infectious hepatitis A virus cDNA to study viral attenuation".

Peter Collins

Invited speaker, Gene Transfer and Expression Conference, Chapel Hill, N.C., October 26-29, 1986.

Invited speaker, The Upjohn Company, Kalamazoo, MI., January 8, 1987.

Invited speaker, NIAID-WHO workshop on Respiratory Syncytial and Parainfluenza Type 3 Viruses, May 4-5, 1987.

Workshop Chairperson, American Society for Virology, 1987 annual meeting, Chapel Hill, NC, May 31-June 4, 1987.

Invited speaker, American Society for Virology, 1987 annual meeting, Chapel Hill, NC, May 31,-June 4, 1987.

Jorge Flores

Invited speaker, Foundation for Research on Gastrointestinal Diseases in Childhood (ENGAST) and the Polar Foundation, Caracas, Venezuela, May 4, 1987, "Diarrheal diseases in childhood, the promise of vaccine development".

Yasutaka Hoshino

Invited participant, Working conference, U.S. and Japanese Viral Diseases Panels, U.S.-Japan Cooperative Medical Sciences Program in Nagasaki, Japan, November 17-20, 1986.

Philip Johnson

Invited speaker, NIAID-WHO workshop on Respiratory Syncytial and Parainfluenza Type 3 Viruses, May 4-5, 1987.

Ruth Karron

Invited speaker, International Symposium on Viral Hepatitis, London, Eng., May 25-28, 1987.

Lewis Markoff

Invited speaker, Modern Approaches to New Vaccines Including the Prevention of AIDS meeting, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, Sept. 9-13, 1987, "Antigenic analysis of the dengue envelope glycoprotein using synthetic peptides".

Roger Miller

Invited speaker, Eighth U.S.-Japan Hepatitis Panel Meeting, Otsu, Japan, March 12-13, 1987.

Invited speaker, Institute of Virology, Chinese Academy of Preventive Medicine, Beijing, Peoples Republic of China, March 18, 1987.

Invited speaker, Anti-Epidemic Center of GuangXi, Nanning, Peoples Republic of China, March 25, 1987.

Invited speaker, International Symposium on Viral Hepatitis, London, Eng., May 25-28, 1987.

Robert Olmsted

Invited speaker, NIAID-WHO workshop on Respiratory Syncytial and Parainfluenza Type 3 Viruses, May 4-5, 1987.

Invited speaker, Annual Meeting of the American Society for Virology, University of North Carolina, Chapel Hill, NC. "Analysis of respiratory syncytial virus gene expression by recombinant vaccinia viruses", May 31-June 4, 1987.

Invited speaker, Modern Approaches to New Vaccines Including the Prevention of AIDS meeting, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, Sept. 9-13, 1987, "Evaluation in non-human primates of the safety immunogenicity and efficacy of recombinant vaccinia viruses expressing the F and G glycoproteins of respiratory syncytial virus".

Gregory Prince

Invited speaker, NIAID-WHO workshop on Respiratory Syncytial and Parainfluenza Type 3 Viruses, May 4-5, 1987.

Mark Snyder

Invited speaker, Modern Approaches to New Vaccines Including the Prevention of AIDS meeting, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, Sept. 9-13, 1987, "Identification of four genes which attenuate influenza A/Ann Arbor/6/60 (H2N2) cold adapted reassortant viruses".

Melanie Spriggs

Invited speaker, NIAID-WHO workshop on Respiratory Syncytial and Parainfluenza Type 3 Viruses, May 4-5, 1987.

Koki Taniguchi

Invited speaker, VIIth International Congress of Virology, Edmonton, Alberta, Canada, August 9-16, 1987, "Localization of cross-reactive and serotype-specific neutralization epitopes on human rotavirus VP3 and VP7".

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00308-06 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
In Vitro and In Vivo Studies of Hepatitis A Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ruth A. Karron, M.D.	Guest Researcher, HV Section	LID, NIAID
Others:	Stephen M. Feinstone, M.D.	Medical Officer	LID, NIAID
	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID

COOPERATING UNITS *(if any)*
Smith-Kline-RIT (Dr. d'Hondt); Office of Biologics, FDA (Dr. Daemer)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Hepatitis Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.2	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hepatitis A virus has been successfully adapted to growth in African green monkey kidney tissue culture. The virus is predominantly cell-associated and does not produce cytopathic effects (CPE). It was attenuated for chimpanzees after 10 or 20 serial tissue culture passages. The attenuated virus has been shown not to revert to virulence during serial passage through chimpanzees. The tissue culture-adapted HAV has been thrice cloned and clones representing passages 21 and 32 have been characterized in chimpanzees and marmosets and compared with wild-type virus. Immunization of chimpanzees with the attenuated virus protected all vaccinated animals from hepatitis A following challenge with wild-type HAV.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00309-06 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structural and Antigenic Analysis of Hepatitis A Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Stephen M. Feinstone, M.D.	Medical Officer	LID, NIAID
Others:	Robert H. Purcell, M.D.	Head, HA Sect.	LID, NIAID
	Ruth Karron, M.D.	Guest Researcher	LID, NIAID
	Jeff Cohen, M.D.	Medical Staff Fellow	LID, NIAID

COOPERATING UNITS *(if any)* NIAID, LIG (Dr. Maloy); Scripps Inst., La Jolla, California (Dr. R. Lerner), University of North Carolina School of Medicine (Dr. Stanley Lemon).

LAB/BRANCH
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SECTION
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INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Antibodies to hepatitis A virus structural proteins have been produced in guinea pigs by inoculating either SDS disrupted purified hepatitis A virus or in rabbits by inoculating synthetic peptides. These antibodies have been used to study the structural proteins of hepatitis A virus by Western blot and the antigens of the virus by in vitro neutralization and radioimmunoassays. Results indicate that the VP0 cleavage into VP2 and VP4 does occur but it is not known if VP4 remains a part of the mature virion. Neutralizing epitopes have been sought by testing antibodies to synthetic oligopeptides of VP1. Only one of these antibodies neutralizes at a very low level. Two epitope libraries have been constructed in Lambda gt 11. No clones synthesizing a peptide that could be indentified by a neutralizing monoclonal antibody have been identified. It is concluded that the neutralization epitopes are probably conformational.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00311-06 LID

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Search for New Hepatitis Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John Ticehurst, M.D.	Medical Officer	LID, NIAID
Others:	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID
	Albert Kapikian, M.D.	Head, Epidemiology Sect.	LID, NIAID

COOPERATING UNITS *(if any)* CC Dept Transf Med, NIH (Alter); Nat Inst Virol, Pune, India (Pavri); Med College, Srinagar, India (Khuroo); Mt Sinai Hosp, NYC (Popper); Inst Pasteur d'Algerie, Algiers, (Belabbes); Ivanovsky Inst, Moscow, USSR (Zhdonov); Walter Reed Army Inst Res (Burke); Washington VAMC (Sjogren)

LAB/BRANCH

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SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	0.8	1.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

New hepatitis agents continue to be recognized. Recently, a form of epidemic hepatitis occurring in India was found not to be caused by any of the recognized hepatitis viruses. Previous attempts to transmit an agent from acute-phase clinical samples to primates were partially successful. Efforts to identify viral agent(s) in recently obtained clinical specimens from India, Algeria and the USSR include primate transmission studies, immune electron microscopy, and cloned cDNA hybridization. Transient low-level liver enzyme elevations and histopathologic changes consistent with hepatitis in liver biopsies were observed in some animals, but this was not uniform, and attempts to transmit an agent serially in chimpanzees and marmosets also produced irregular results. Although an etiologic agent has not been identified in experimental animals and none of the specimens has hybridized with probes that detect a wide range of picornaviruses including HAV, several different virus-like particles have been visualized. One of these particles, in feces from an Indian patient, is approximately 27 nm in diameter and appears to have surface structure. This particle apparently has a wide geographic distribution because of its reactivity with sera from Algeria, USSR, Pakistan and India. Serologic responses to infection were demonstrated in a naturally infected human and an experimentally inoculated chimpanzee.

LMSF
LMI
LMM
LPD
LVD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00312-06 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Clinical and Experimental Studies of Hepatitis B Vaccines

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	Robert H. Purcell, M.D.	Head, HV Sect.	LID, NIAID
Others:	John Ticehurst, M.D.	Medical Officer	LID, NIAID

COOPERATING UNITS (*if any*) Baylor Sch. Med., Houston, TX (Hollinger); Div. Mol. Virol. & Immunol., G.U., DC (Gerin); Dept. Med., Rutgers Med. Sch., New Brunswick, NJ (Mc-Auliffe); CDC, Atlanta, GA (Francis); Dept. Epid., Shanghai 1st Med. Coll. (Xu).

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Hepatitis Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Clinical testing and characterization of one lot of plasma-derived hepatitis B vaccine prepared by the NIAID have been completed. The vaccine was highly immunogenic, safe and well tolerated when tested in healthy persons ranging from infants to adults. Results from an efficacy trial in Asia indicate that the vaccine effectively prevents transmission of hepatitis B virus infection from mothers to infants. The sera from these infants are being analyzed for responses to specific HBsAg epitopes in an attempt to identify those that are most important.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00314-06 LID

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Woodchuck Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Roger Miller, Ph.D. Senior Staff Fellow, HV Section LID, NIAID

COOPERATING UNITS *(if any)* Div. Molec. Virol. & Immunol., Georgetown U, Washington, DC (Dr. Gerin); New York State College of Veterinary Medicine (Dr. B. Tennant)

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Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda Maryland 20892

TOTAL MAN-YEARS: 3.1	PROFESSIONAL: 2.2	OTHER: 0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The woodchuck hepatitis virus (WHV) is taxonomically and serologically related to the hepatitis B virus (HBV). Infection with each of these viruses is associated with acute and chronic hepatitis and hepatic cell carcinoma in their respective hosts; these associations appear to be etiological in nature. The WHV/woodchuck model system provides a convenient means of studying the relationship between virus and host in the oncogenic process. Tests specific for the WHV antigen-antibody systems have been developed. Genetic relationships between the hepadnaviruses and retroviruses suggest a common origin. Genetically altered WHV genomes, constructed by recombinant DNA techniques are being studied in woodchucks in order to determine the biological significance of individual gene products.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00316-06 LID

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Delta Agent

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID

COOPERATING UNITS *(if any)* Georgetown U., Washington, DC (Gerin); CDC, Phoenix, Arizona (Hadler); Mt. Sinai Hospital, NYC (Popper); Chiba U., Chiba City, Japan (Omata); U. Washington Med. Research Unit, Taipei, Taiwan (Beasley)

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SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The delta agent is a transmissible hepatitis virus that appears to be defective and requires co-infection with hepatitis B virus for its own synthesis. The agent has a small RNA genome ($10^{5.7}$ daltons) that is encapsidated together with delta antigen within a coat of HBsAg. The agent was discovered in 1977 in Italy, where it is endemic. Evidence for infection with the delta agent is found most frequently in carriers of hepatitis B virus who are repeatedly exposed to blood (hemophiliacs, illicit drug users, etc.). Sensitive assays for delta agent infection have been developed and used to evaluate experimental infection of HBV-carrier chimpanzees. In both man and chimpanzee infection with the delta agent results in very severe hepatitis. The delta agent has been experimentally transmitted to woodchucks chronically infected with the woodchuck hepatitis virus, a hepatitis virus similar to hepatitis B virus. The chimpanzee and woodchuck provide animal model systems for more detailed characterization of this medically important agent.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00317-06 LID

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biology of Non-A, Non-B Hepatitis Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Stephen M. Feinstone, M.D.	Medical Officer	LID, NIAID
Others:	Robert H. Purcell, M.D.	Head, HV SEction	LID, NIAID

COOPERATING UNITS *(if any)* Blood Bank, CC, NIH, Bethesda, MD (Dr. Alter, Dr. Shih, Dr. Esteban); CH, LMG, NIH, Bethesda, MD (Dr. Dawid, Dr. Sargent). Nihon U. School Med., Tokyo, Japan (Dr. Y. Shimizu); Cetus Corp., Emeryville, CA. (John Sninsky)

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Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.4	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The agent of non-A, non-B hepatitis continues to resist identification. A selected cDNA library was produced and screened for specific non-A, non-B hepatitis virus or disease related sequences, but none was identified. A second more highly selected library is presently being constructed. A cDNA expression library in λ gt 11 was constructed and screened with Dr. Shimizu's monoclonal antibody. No clones producing an antigen that reacts with the Shimizu antibodies have yet been isolated. The relationship of the non-A, non-B agent to other viruses, especially HBV and HDV, is being investigated using the primer pair amplification system developed at Cetus Corp.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00319-05 LID (Terminated 1987)

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
New Approaches to the In Vitro Propagation of Non-Cultivatable Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID

COOPERATING UNITS *(if any)*
Office of Biologics, FDA (Dr. Daemer)

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Laboratory of Infectious Diseases

SECTION
Hepatitis Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

There is a need for an in vitro substrate for the cultivation of hepatitis viruses. Hepatocytes would seem a logical choice, but it is very difficult to obtain and maintain primate hepatocytes in culture. We are attempting to develop hepatocyte-hepatoma hybridomas of primate origin. Such hybrid cells would be expected to have the receptor sites and metabolic systems suitable for synthesis of hepatitis viruses and the ability of hepatoma cells to multiply indefinitely in vitro. Methods for the selection of hybrid cells without drug markers (i.e. use of vital dyes) have been developed. These and other vital dyes are being studied for their ability to detect metabolic changes in cells that are infected with viruses.

TERMINATED 1987

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00321-06 LID

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development, Characterization, and Use of Cloned Hepatitis A Virus (HAV) cDNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John Ticehurst, M.D.	Medical Officer	LID, NIAID
Others:	Stephen M. Feinstone, M.D.	Medical Officer	LID, NIAID
	Robert H. Purcell, M.D.	Head, HV Sect.	LID, NIAID
	Jeffrey I. Cohen, M.D.	Medical Staff Fellow	LID, NIAID
	Manfred Weitz, Ph.D.	Fogarty Visiting Fellow	LID, NIAID
	Ruth A. Karron, M.D.	Guest Worker	LID, NIAID

COOPERATING UNITS *(if any)* G'town U., (Baroudy); Col. U., (Racaniello); U. Neb. (Tracy); U. Wisc. (Palmenberg); U. Turku, Finland (Hyypia); Genentech Corp, CA (Kleid); Scripps, CA (Lerner); LMB, NCI (Maizel); LIG, NIAID (Maloy); Athens Sch. Hyg., Greece (Tassopoulos); AFIP, D.C. (Anderson); LVD, NIAID (Moss, Fuerst)

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Hepatitis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.0	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Wild-type type HAV, strain HM-175 RNA, was transcribed into cDNA and cloned. The cloned cDNA was used as a probe for detecting HAV RNA in tissue culture, serum, and fecal specimens by hybridization. Hybridization experiments also demonstrated that cDNA probes representing the HAV genome will not hybridize to RNA or cloned cDNA from a variety of other picornaviruses. From analysis of the nucleotide and predicted amino acid sequences of this genome, we have concluded that HAV has typical picornaviral genome organization but sequences that are quite different from other picornaviruses. We believe that it should be classified in a separate picornaviral genus. HAV VPg was demonstrated by immunoprecipitation. Encephalomyocarditis virus and vaccinia virus expression systems are being used in attempts to express the structural and nonstructural proteins of HAV. As an extension of hybridization studies with cloned HAV cDNA, RNA probes representing coxsackievirus sequences are being used in attempts to detect coxsackieviruses in infected tissue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00370-05 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of Acquired Immune Deficiency Syndrome (AIDS)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert H. Purcell, M.D.	Head, HV Section	LID, NIAID
Others:	Philip Johnson, M.D.	IPA (Vanderbilt)	LID, NIAID

COOPERATING UNITS *(if any)* NINCDS, NIH, Bethesda, MD (Dr. London); Gallo); LIG, NIAID, NIH, Bethesda, MD (Dr. Folks, Dr. Fauci); Georgetown U. (Dr. Gerin).

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Laboratory of Infectious Diseases

SECTION
Hepatitis Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A Zaiairian isolate of human immunodeficiency virus (HIV) designated Z-34 was tested for relative infectivity titer in vitro and in chimpanzees. The titer of the virus pool in cell culture was approximately 10^4-10^5 tissue culture infectious doses (TCID) ml. However, the infectivity titer in chimpanzees was much lower. The reason for this discrepany may be related to previous infections of the chimpanzees with hepatitis viruses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00404-04 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Second and Third-Generation Hepatitis B Vaccines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Robert H. Purcell, M.D. Head, HV Section LID, NIAID

COOPERATING UNITS *(if any)* Georgetown U. Washington, DC (Dr. Gerin); Scripps Institute, La Jolla, California (Dr. Lerner); LBV, NIAID, NIH, Bethesda, MD (Dr. B. Moss)

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SECTION
Hepatitis Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Expense, availability and/or incomplete acceptance based upon an unfounded fear of infection with the agents of AIDS limit the impact of plasma-derived hepatitis B vaccines in developed and developing countries. There is therefore a need for new approaches to vaccine development. Recombinant DNA technologies appear to offer the best opportunities for the next generation of hepatitis B vaccines. Partial or complete protection against hepatitis B has been demonstrated in chimpanzees following vaccination with (a) recombinant derived subunit vaccine prepared in eukaryotic cells, (b) live recombinant vaccinia virus containing HBV genes, and (c) synthetic peptides representing HBsAg sequences. Attempts to identify the antigenic domains most important in stimulating neutralizing antibody are currently in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00405-04 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Inactivation of Hepatitis Viruses in Pooled Plasma Derivatives

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Robert H. Purcell, M.D. Head, HV Section LID, NIAID

COOPERATING UNITS *(if any)* Georgetown U., Washington DC (Dr. Gerin); Southwest Fndt., San Antonio, TX (Dr. Eichberg); Revlon Inc., Tuckahoe, NY (Dr. Landaburu)

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Laboratory of Infectious Diseases

SECTION
Hepatitis Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The transmission of viral hepatitis by plasma derivitives continues to be an important medical problem that has been made more urgent by the similar epidemiology of the agent of acquired immune deficiency syndrome. Methods of inactivating viruses in plasma derivatives while retaining the biological potency of the products are being explored. These include the extraction of lyophilized or aqueous preparations with lipid solvents such as chloroform.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00406-04 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Structure of Hepatitis A Virus (HAV): Genome Sequence and Organization

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Jeffrey I. Cohen, M.D. Medical Staff Fellow LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Hepatitis Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 0.9	PROFESSIONAL: 0.4	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The complete nucleotide sequence of wild-type hepatitis A virus (HAV) strain HM-175 has been determined. Comparison of wild-type HAV HM-175 with a different cell culture-adapted, HAV strain (Najarian, PNAS 1985) revealed 624 nucleotide differences (91.7% identity), but only 34 amino acid differences (98.5% identity).

The complete nucleotide sequence of an attenuated, candidate vaccine derivative of HAV HM-175 was determined. Comparison of the nucleotide sequence of cDNA from wild-type virus and the attenuated HAV mutant revealed 24 nucleotide changes. Seven nucleotide changes, including five base deletions, occurred in the 5' noncoding region. Sixteen nucleotide changes, including 12 amino acid changes, occurred in the coding region. Two amino acid changes were present in the capsid proteins and one of these resulted in a change in the charge of an amino acid in VP1, thought to be the immunodominant capsid protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00440-03 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Construction of Full Length Hepatitis A Virus cDNA for Transfection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Jeffrey I. Cohen, M.D. Medical Staff Fellow LID, NIAID

COOPERATING UNITS *(if any)*

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Laboratory of Infectious Diseases

SECTION
Hepatitis Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 0.7	PROFESSIONAL: 0.4	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Full length wild-type HAV cDNA and its RNA transcripts failed to produce HAV when transfected into marmosets or monkey kidney cells. In contrast, a full length cDNA of an attenuated, cell culture-adapted mutant of HAV which was recently molecularly cloned proved to be infectious when transfected into monkey kidney cells. Transfection with RNA transcripts produced in vitro from the infectious cDNA yielded about 10-fold more HAV than transfection with the cDNA itself. HAV produced during transfection was then inoculated into marmosets. The animals developed liver enzyme patterns that closely resembled the enzyme patterns seen in marmosets inoculated with virus with a comparable level of cell culture passage.

Chimeric cDNAs which contain portions of both wild-type and attenuated HAV cDNA were constructed. RNA transcripts from several of these cDNA constructs were infectious when transfected into monkey kidney cells. These viruses will be inoculated into marmosets to determine which portions of the attenuated HAV genome are associated with attenuation. In addition, site-directed mutagenesis is being used to construct cDNAs with selected point mutations and these mutants will be analyzed by transfection in an attempt to identify the molecular basis for cell culture adaptation and attenuation as as well as other specific viral functions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00473-02 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Replication of Hepatitis A Virus In Vivo

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Jeffrey I. Cohen, M.D. Medical Staff Fellow LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Hepatitis Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 0.5	PROFESSIONAL: 0.4	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Extra-hepatic sites of hepatitis A virus replication have been examined during experimental infection. A chimpanzee was inoculated orally with wild-type HAV HM-175 and seroconverted to HAV 23 days after inoculation. Serum, saliva, and throat swabs were inoculated into seronegative marmosets. HAV was present in the serum 18 days after inoculation, in saliva 23 days after inoculation, and in throat swabs 18 to 21 days after inoculation. Additional specimens will be tested using cDNA hybridization and fluorescein-labeled antibody.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00323-06 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure and Expression of Parainfluenza Type 3 Virus Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Melanie K. Spriggs, Ph.D.	Staff Fellow	LID, NIAID
	Robert A. Olmsted, Ph.D.	Staff Fellow	LID, NIAID
	Philip R. Johnson, M.D.	IPA (Vanderbilt U.)	LID, NIAID
	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID

COOPERATING UNITS *(if any)*
NINCDS, NIH (Dr. William T. London)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Respiratory Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.2	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

cDNA clones and complete nucleotide sequences are now available for five of the six mRNAs of human parainfluenza virus type 3 (PIV3). Amino acid sequence homologies and similarities in gene map were examined to investigate relationships between PIV3 and other paramyxoviruses. Among other things, this provided evidence for a close evolutionary relationship between PIV3 and Sendai virus (murine parainfluenza virus type 1) and a more distant relationship with simian virus 5 (canine parainfluenza virus type 2). Recombinant vaccinia viruses were constructed that expressed F, HN or NP protein that appeared to be authentic biochemically, biologically and immunologically. Immunization of cotton rats with either vaccinia-F or vaccinia-HN or both induced a high level of PIV3-specific serum neutralizing antibodies and immunized animals exhibited significant resistance to challenge with PIV3. This established that each of the two glycoproteins is a major neutralization antigen, although HN appeared to play the greater role in protective immunity. Immunization of nonhuman primates (patas monkeys) with vaccinia F and vaccinia HN induced a high level of serum neutralizing antibodies and immunized animals exhibited significant protection from PIV3 challenge in both the upper and lower respiratory tract. This was the first demonstration that a single parenteral inoculation of a recombinant virus can induce immunity in primates against a respiratory tract virus. The HN cDNA also was expressed in biologically active form with a late-replacement SV40 vector. Site directed mutagenesis was used to investigate the roles of specific polypeptide domains and amino acid residues in intracellular transport of HN and in neuraminidase and hemagglutination activities.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00324-06 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Laboratory Studies of Myxoviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mark H. Snyder, M.D.	Senior Staff Fellow	LID, NIAID
Others:	Alicia Buckler-White	Staff Fellow	LID, NIAID
	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(if any)*
U. of Michigan (Dr. Maassab, Dr. DeBorde)

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Laboratory of Infectious Diseases

SECTION
Respiratory Viruses Section

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NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies with reassortant viruses which contained human influenza A virus surface antigens from the A/Washington/80 (H3N2) virus and one or more internal genes from the avian A/Pintail/Alberta/79 virus demonstrated that two specific constellations of polymerase genes specify a host-dependent restriction of replication in MDCK but not chick kidney tissue culture. The constellation associated with the higher degree of host range restriction in vitro also specified restriction of replication in the respiratory tract of squirrel monkeys. A reassortant virus containing this constellation of genes that restrict replication of the virus in primates could be used as a donor of this attenuating gene constellation to new influenza virus variants as they appear in nature.

These studies also demonstrated that the M gene of the avian influenza A/Pintail/79 virus does not restrict viral replication in the lower respiratory tract of monkeys. Analysis of the nucleotide sequence of the A/Pintail/79 virus and comparison of the deduced amino acid sequence with that of the avian influenza A/Mallard/NY/78 virus M gene which does specify attenuation revealed only four amino acid changes, three of which are conservative. None of these amino acid changes occurred at the previously defined sites of host species specific amino acid substitutions. These observations suggest that a single amino acid change may be responsible for the attenuation specified by the A/Mallard/NY/78 virus M gene.

Evaluation of single gene substitution human influenza A/Ann Arbor/60 ca x A/Korea/1/82 reassortant viruses was completed. Our results demonstrated that the ca donor virus PA gene specifies the ca phenotype, the PB1 and PB2 genes specify the ts phenotype, and four genes PB1, PB2, PA and M may independently contribute to attenuation of ca reassortant viruses in humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI00325-06 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of Respiratory Viruses in Primates.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
Others:	Mark H. Snyder, M.D.	Senior Staff Fellow	LID, NIAID
	Kathleen L. Coelingh, Ph.D.	Senior Staff Fellow	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID
	Melanie Spriggs, Ph.D.	Staff Fellow	LID, NIAID
	Robert Olmsted, Ph.D.	Staff Fellow	LID, NIAID
	Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS *(if any)* Meloy Laboratories, Rockv., MD (J. Philips); NINCDS, NIH, (Wm.T. London); LVD, NIAID, NIH (B. Moss, R.M. Buller); Dept. Pediatrics, USUHS, Beth., MD (V. Hemming); Johns Hopkins U. Schl. Public Health, Balti., MD (G. Prince)

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	0.5	0.8

CHECK APPROPRIATE BOX(ES)
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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

(1) Reassortant viruses containing a specific polymerase constellation of PB2 and PA genes of the human influenza A/Washington/80 virus and the PB1 derived from the avian influenza A/Pintail/79 virus were completely restricted in replication in the respiratory tract of squirrel monkeys. The M gene of the avian influenza A/Mallard/78 virus but not that of the avian influenza A/Pintail/79 virus, also specified restriction of viral replication in monkeys. The amino acid sequences of these two M genes differ by only 4 amino acids suggesting that one or more of these amino acids is involved in host range restriction of replication specified by the A/Mallard/78 M gene in primates. (2) A bovine parainfluenza virus type 3 (PIV3) was isolated in a cell line acceptable for use in a human vaccine. This strain was significantly restricted in replication in the upper and lower respiratory tract of rhesus monkeys suggesting that it may be attenuated for humans. Despite its restricted replication in monkeys the bovine PIV3 induced moderate levels of antibody to the human PIV3. These findings provide a basis for evaluating the bovine PIV3 virus in humans for its safety and efficacy in the prevention of illness due to human PIV3. (3) Human immune gamma globulin (IgG) with a high titer of neutralizing antibodies to RSV was administered into the trachea of RSV-infected owl monkeys. Topical immunotherapy at the height of viral infection reduced the level of RSV replication in the lungs. (4) Vaccinia-recombinant viruses containing the gene for the hemagglutinin-neuraminidase (HN) glycoprotein or the fusion (F) glycoprotein of the human PIV3 stimulated a high titer of neutralizing antibodies in patas monkeys and induced a high level of resistance to PIV3 challenge in both the upper and lower respiratory tract. A vaccinia virus recombinant containing the gene for the attachment (G) or fusion (F) glycoprotein of respiratory syncytial virus induced a level of neutralizing antibodies in owl and patas monkeys that had previously been associated with re-sistance to RSV infection in man and cotton rats.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00326-06 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of Respiratory Viruses in Volunteers.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mark H. Snyder, M.D.	Senior Staff Fellow	LID, NIAID
Others:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(if any)* Flow Labs, Rockv. MD (Potash); Johns Hop. U. Balt. MD (Clements, Sears,Steinhoff,Halsey); U Roch. S.Med.Roch. NY (Betts,Dolin); Vanderbilt U S. Med.Nashv.TN (Wright); Marshall U.S.Med. Huntington, WVA (Belshe); FDA,Beth.MD (Burlington); NCI/NIH,Beth.MD (Wagner,Nelson)

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	0.3	0.9

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A side-by-side comparison of the safety, attenuation, infectivity, immunogenicity, and efficacy of avian-human (AH) influenza A/Mallard/NY/6750/78 and *ca* A/Ann Arbor/6/60 reassortant viruses was performed. Reassortant viruses derived from the wild-type human A/Bethesda/1/85 (H3N2) or A/Texas/1/85 (H1N1) virus were evaluated in seronegative volunteers some of whom were subsequently challenged with wild type virus. The results showed that the AH and *ca* reassortant viruses were similar in their level of attenuation, immunogenicity, infectivity, and efficacy. Studies have been iniatiated to systematically evaluate the responses of seronegative children to AH and *ca* reassortant H1N1 and H3N2 virus vaccines.

Studies with reassortant viruses which contain a single RNA segment derived from the human influenza A/Ann Arbor/6/60 *ca* donor virus and all other RNA segments from the human wild type influenza A/Korea/1/82 virus demonstrated that two genes of the *ca* donor virus, the PA polymerase gene and the M gene, play a major role in the attenuation of *ca* reassortant viruses for humans. The PB1 and PB2 genes of the *ca* donor virus probably also contribute to attenuation in the lower respiratory tract. These studies provide a biologic basis for the observed stability of *ca* reassortant virus vaccines in humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00327-06 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characterization of Parainfluenza Type 3 Virus Surface Glycoproteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kathleen L. vanWyke Coelingh, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
	Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS *(if any)* Texas A & M Univ. (M. Summers); NCI-Navy Medical Oncology Branch (J. Battey and A.-M. Legacq-Verheyden).

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to expand our previous antigenic and functional analyses of the human parainfluenza type 3 virus (PIV3) HN protein to include structural analysis, we have produced baculovirus recombinants expressing PIV3 HN protein. The HN protein produced by these recombinants in insect cells is glycosylated, transported to the cell surface, biologically active, immunogenic, and antigenically authentic. These properties indicate that the recombinant HN should be a suitable substrate for structural analysis. In addition, this material may prove to be a useful immunogen in a PIV3 vaccine.

To examine the molecular epidemiology of PIV3, we sequenced the HN genes of six clinical PIV3 strains isolated over a 27-year period. Most of the nucleotide variability is found in the 5' untranslated regions, and most of the amino acid divergence is found at the N- and C-terminus of the HN. Variability among HN proteins reflects genetic heterogeneity rather than progressive evolution of PIV3. The antigenic and sequence analysis of the clinical strains indicated that only one serotype of PIV3 exists, a finding that should make the development of an immunoprophalaxis strategy easier.

We have evaluated the bovine PIV3 as a potential live virus vaccine against human PIV3. Bovine PIV3 is attenuated in higher primates. This virus induces an immune response to human PIV3 and immunized primates exhibit resistance to challenge with the human PIV3 strain. A strain of bovine PIV3 suitable for use in humans was identified and found to be satisfactorily attenuated and immunogenic in monkeys.

Monoclonal antibodies to the fusion (F) protein of PIV3 have been produced and are being characterized prior to selection of antigenic variants, epitope mapping, and sequence analysis of the variants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00344-06 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunologic Factors in Respiratory Syncytial Virus (RSV) Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Brian R. Murphy, M.D.	Head, RVS	LID, NIAID
Others:	Robert M. Chanock, M.D.	Chief	LID, NIAID
	Gregory A. Prince, D.D.S., Ph.D.	Guest Worker	LID, NIAID

COOPERATING UNITS *(if any)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.5	0.5

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Potentiation of RSV disease was observed in cotton rats (Sigmodon hispidus) previously immunized with formalin-inactivated RSV. This potentiation of disease resembled that observed 20 years ago during a clinical trial of formalin-inactivated RSV vaccine in young infants. Animals immunized with the Lot 100 RSV vaccine used in the clinical trials of 20 years ago, or with freshly-prepared, formalin-killed RSV, were challenged intranasally with RSV. Accentuated pulmonary pathology developed when these animals were infected with RSV. This potentiated response was characterized by polymorphonuclear and lymphocytic infiltration of the lungs, which began one day after viral challenge. Analysis of this phenomenon indicated that IgG and C3 were deposited in both the lungs and kidneys. Also pulmonary pathology was less severe in complement-depleted animals. Significantly, a dose-response effect was observed which was similar to that described for experimental immune complex disease. These observations suggested that the potentiated disease caused by formalin-inactivated RSV vaccine was in part the result of a pulmonary Arthus reaction.

Efforts begun 8 years ago in our laboratory have recently yielded an inbred strain of cotton rat, which can now be used for studies of the cellular basis of vaccine-induced disease potentiation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00345-06 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunity to RS Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Brian R. Murphy, M.D.	Head, RVS	LID, NIAID
Others:	Robert M. Chanock, M.D.	Chief	LID, NIAID
	Gregory Prince, D.D.S., Ph.D.	Spec. Volunt.	LID, NIAID

COOPERATING UNITS *(if any)*
USUHS, Bethesda, MD (V. Hemming); Childrens Hospital National Medical Center, Wash., D.C. (Rodriguez).

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.4	0.5

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☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A double-blind, placebo-controlled intravenous immunoglobulin (IVIG) immunotherapy study was performed in 35 infants hospitalized with RSV disease. The treatment was well tolerated, resulted in significant reduction in nasal RSV shedding, and significant improvement in transcutaneous oximetry readings. However, the mean duration of hospitalization was not reduced by IVIG treatment. Followup to date has revealed no harmful effects resulting from immunotherapy of RSV infections.

IV1G administered topically offered a significant advantage over systemically administered IVIG, effecting near-complete clearance of pulmonary RSV in cotton rats at a dose 160-fold less than that required for the same therapeutic effect with parenterally administered IVIG. The effect was permanent, and was not due to in vitro viral neutralization during homogenization of pulmonary tissue. Topically administered IVIG, when given prophylactically, provided significant resistance to pulmonary RSV infection for up to seven days, suggesting that there is a specific receptor for IgG in the lungs.

A subunit vaccine consisting of F and G glycoproteins of RSV was highly immunogenic, inducing complete resistance to pulmonary RSV infection in cotton rats at a dose as low as 800 picograms of each protein per immunization. At lower doses of F and G, some animals showed peribronchiolar lymphocytic infiltration following RSV challenge, but infiltration of neutrophils was not observed.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00368-05 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structural Analysis of Respiratory Syncytial Virus Genome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Philip R. Johnson, M.D.	IPA (Vanderbilt U.)	LID, NIAID
	Robert A. Olmsted, Ph.D.	Staff Fellow	LID, NIAID
	Melanie K. Spriggs, Ph.D	Staff Fellow	LID, NIAID

COOPERATING UNITS *(if any)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	1.6	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Human respiratory syncytial virus (RSV) exists as two antigenic subgroups A and B. Previously, for the subgroup A strain A2, we cloned and sequenced nine of the ten viral genes (excluding only the polymerase L gene) and identified the gene map. These studies have now been extended to the subgroup B strain 18537. cDNAs for the 18537 strain are available for nine of the ten genes (excluding the L gene). Comparison with the A2 sequences showed that (i) deduced amino acid sequences and nucleotide sequences of open reading frames were highly conserved (76-96% identity at the amino acid level) except for the G gene and its protein (53% at the amino acid level). (ii) Putative transcriptive nucleotide sequence signals were highly conserved. (iii) Noncoding gene and intergenic sequences exhibited little conservation. (iv) The putative extracellular domain of the G protein exhibited little conservation (44%) identity) except for a short circumscribed region that was highly conserved and thereby is a candidate for a role in receptor binding. The sequence heterogeneity in the extracellular domain of G might be a mechanism for evading host immunity. The ability of G to sustain extensive and rapid (relative to the other RSV proteins) amino acid substitutions without loss of function implied that its higher order structure might be relatively flexible and simple. Sequence analysis is continuing for the genomic termini and L gene of the A2 strain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00372-05 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
RSV Proteins: Roles in Host Immunity and Immunoprophylaxis.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Robert A. Olmsted, Ph.D.	Staff Fellow	LID, NIAID
	Philip R. Johnson, M.D.	IPA (Vanderbilt U.)	LID, NIAID
	Melanie K. Spriggs, Ph.D.	Staff Fellow	LID, NIAID
	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(if any)* NINCDS, NIH (London); LVD, NIAID, NIH (Moss, Buller); Johns Hopkins U. Sch. of Pub. Hlth, Balt., MD (Prince); Wistar Inst., Phila., PA (Yewdell); U. Roch. Sch. Med., Roch. NY (Walsh); MicroGeneSys Inc., West Haven, CT (Cochran, Smith, Ericson).

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The antigenic relationships between the F and G proteins of the two RSV antigenic subgroups were compared using RSV-vaccinia virus recombinants and glycoprotein-specific ELISA. These studies showed that the F protein was highly conserved antigenically whereas the G protein exhibited significant antigenic heterogeneity. Recombinant RSV-vaccinia viruses were constructed for the 1C, 1B, N, P, M, 1A and 22K proteins to investigate processing, functions and contribution to host immunity of the individual RSV proteins. The F and N proteins, but not the 1A and G proteins, were major targets for RSV-specific murine cytotoxic T cells. Immunization of cotton rats with the 1A or N protein did not induce a significant level of protective immunity. The F and G proteins have been expressed in insect cells by recombinant baculoviruses to use as a potential subunit vaccine and as an additional tool in investigating protein processing.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00457-03 LID

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Human Immune Response to Respiratory Syncytial Virus Infection or Vaccination

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principel Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
Others:	Gregory Prince, D.D.S.,Ph.D.	Spec. Volunteer	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID
	Mark H. Snyder, M.D.	Senior Staff Fel.	LID, NIAID

COOPERATING UNITS *(if any)* U. Roch. Sch. Med. Roch., N.Y. (Walsh); Ch. Hosp. Nat. Med. Ctr. D.C. (Kim, Parrot); NCI/NIH, Beth. MD. (Nelson, Wagner); CDC Atlanta, GA (Reimer); BOB, FDA, Beth. MD. (Hendry); U. N. Carolina, Chapel Hill, N.C. (Henderson); Karolinska Institut, Stockholm, Sweden (Norrby);OSD/NIAID (Alling)

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

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☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In infants and young children formalin-inactivated RSV vaccine induced a high level of binding (ELISA) antibodies to the RSV F glycoprotein but these antibodies were deficient in both neutralizing and fusion-inhibiting activity. Radioimmunoprecipitation of post-immunization sera revealed that antibodies to the internal viral M and NP antigens were also produced by a majority of the vaccinees tested. Thus, formalin-inactivated vaccine induced high levels of antibodies to the M and NP antigens of RSV which are not involved in protective immunity. These antibodies as well as the non-functional antibodies to the viral surface glycoproteins may have acted in concert to produce a pulmonary Arthus reaction which contributed significantly to the potentiation of disease that occurred when vaccinees were infected with RSV.

Immunization of infants and children who had not been infected with RSV previously led to a potentiated disease in 70 to 80% of these vaccinees, whereas immunized individuals who had been infected previously had a much lower frequency of disease potentiation.

The IgG subclass antibody response to the F and G glycoproteins of RSV of infants and children undergoing first, second, and third infection with RSV revealed that the IgG_1 response to the G glycoprotein was deficient and that the IgG_2 response to the heavily glycosylated G glycoprotein was neither age dependent nor different from that to the F glycoprotein.

The antigenic relatedness of the RSV subgroup A and subgroup B strains was analyzed using sera from infants who had a primary infection with RSV. The F glycoproteins of subgroup A and subgroup B strains were highly related, whereas the G glycoproteins were only 5% related. Relatedness between subgroups was 30% when analyzed by cross-neutralization.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00474-02 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Mechanisms Underlying Adenovirus Pathogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Harold S. Ginsberg, M.D.	IPA	LID, NIAID
Others:	Robert M. Chanock, M.D.	Chief	LID, NIAID
	Gregory Prince,	Guest Worker	LID, NIAID
	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
	Kim Green, Ph.D.	Staff Fellow	LID, NIAID

COOPERATING UNITS *(if any)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In Sigmadon hispidus cotton rats types 2 and 5 adenoviruses produce diffuse pneumonia consisting of peribronchiolar and alveolar septal monocytic infiltration. Detailed studies with type 5 adenovirus show that the extent of pneumonia and degree of viral replication is strictly dose dependent. Utilizing type 5 ts mutants unable to replicate viral DNA at the non-permissive it was shown that only early viral events are essential to effect the pneumonic infiltrations. Ela deletion mutants did not cause the pneumonic infiltrations, but the block in transcriptions from all other early regions made interpretation of this finding difficult. However, Elb mutants that produce a truncated 55K protein replicate like wild-type (Wt) virus but cause very minimal pneumonia. Deletion of the 5' half of the E3 region, which encodes a 19K glycoprotein, produces more extensive pneumonia than Wt virus. The mechanism(s) producing this increase may require products of more than a single open reading frame (ORF) in E3.

The pathology and replication of wild-type and vaccine strains of type 4 adenovirus are being studied following intranasal inoculation or oral ingestion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00497-01 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characterization of the Fusion Glycoprotein of Respiratory Syncytial Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kathleen L. van Wyke Coelingh, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Judith A. Beeler, M.D.	IPA	LID, NIAID
	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
	Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID

COOPERATING UNITS *(if any)* Karolinska Inst., Stockholm, Sweden (Norrby); CDC, Atlanta, GA (Anderson); BOB, Bethesda, MD (Hendry); Georgetown U., Washington, D.C. (Fernie); Children's Hosp. Nat. Med. Center, D.C. (Kim)

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have produced a panel of neutralizing and non-neutralizing mAbs specific for the F glycoprotein of the A2 strain of RSV. Some of the neutralizing mAbs have enhanced neutralizing activity in the presence of complement and one mAb requires complement for virus neutralization. Most neutralizing mAbs also inhibit fusion activity, but there is no strict correlation between high neutralizing activity and high fusion-inhibition activity. Our panel of neutralizing mAbs recognizes a minimum of 4 distinct epitopes, which appear to be distribu-ted within 3 antigenic sites. The total number of F epitopes recognized by our panel will be known when analyses of their reactivity patterns with laboratory-selected monoclonal antibody-resistant mutants (MARMs) and RSV clinical strains is completed. When this information is completed we will analyze the F gene sequences of the MARMs to determine the amino acid residues involved in each of the F epitopes.

Several of the MARMs are also phenotypic variants, exhibiting a lytic form of cytopathology rather than syncytium-formation. When the amino acid residues responsible for these phenotypes are identified, we will begin to understand the relationship between the molecular structure of the F glycoprotein and its biological functions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00498-01 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Synthesis, Processing and Functions of the Proteins of Human RSV.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID
Others:	Robert A. Olmsted, Ph.D.	Staff Fellow	LID, NIAID
	Melanie K. Spriggs, Ph.D.	Staff Fellow	LID, NIAID
	Philip R. Johnson, M.D.	IPA	LID, NIAID

COOPERATING UNITS *(if any)*
LVD, NIAID, NIH (B. Moss); LIG, NIAID, NIH (W. Lee Maloy)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Respiratory Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	0.7	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The structure and processing of the small 1A protein and attachment G glycoprotein of human respiratory syncytial virus (RSV) were investigated using recombinant viral vectors, site-directed mutagenesis and peptide-specific antisera. The 1A protein was shown to be a third integral membrane protein of RSV. The 1A protein was processed intracellularly by a multi-step pathway that included glycosylation, and appeared in abundance at the cell surface. Site directed mutagenesis was used to construct a series of G protein mutants that contained COOH-terminal truncations of increasing length. Expression of these was used to map protein domains that are involved in the addition of O-linked sugar and in cell-surface transport. These G mutants might be useful in mapping antigenic sites.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00332-05 LID
(Terminated 1987)

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Engineering the Genome of Influenza Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
Others:	Lewis J. Markoff, M.D.	Medical Officer	LID, NIAID
	Kevin Ryan, Ph.D.	Staff Fellow	LID, NIAID
	Erich Mackow, Ph.D.	Staff Fellow	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(if any)*
The Wistar Inst., Philadelphia, Pa. (Jonathan Yewdell)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our goal has been to use recombinant DNA techniques to construct influenza virus mutants with deletions in strategic regions of the genome. Viable deletion mutants would be especially valuable for use in immunoprophylaxis since these mutants would be unlikely to revert and therefore should be stable as regards phenotype. With this goal in mind, we produced full-length cloned DNA sequences of gene segments of an H3N2 influenza A virus. Thus far we have cloned and characterized 6 full-length genes (hemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), matrix protein (M), non-structural proteins (NS), and polymerase protein PB2): the remaining two genes (PB1 and PA) have also been cloned but not in complete form. These full-length DNA clones should produce corresponding RNA transcripts that contain the control sequences needed for transcription and replication of viral genes. The validity of this prediction was established for transcription and expression of viral proteins. Functional influenza viral protein (HA, NA, or NP) was produced when simian cells were transfected with a SV40 recombinant vector containing cloned influenza DNA. The influenza cDNA was inserted into the late region of SV40 in an orientation which resulted in transcription of (+) strand influenza RNA. Attempts to rescue cloned influenza DNA by coinfection of transfected cells with influenza A virus were unsuccessful.

TERMINATED 1987

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00365-04 LID (Terminated 1987)

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Functional Analysis of Signal Sequences of Influenza Virus Hemagglutinin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
Others:	Lewis Markoff, M.D.	Medical Officer	LID, NIAID
	Alicia Buckler-White, Ph.D.	Staff Fellow	LID, NIAID
	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID

COOPERATING UNITS *(if any)*
Laboratory of Immunogenics, NIAID, NIH, Bethesda, MD. (Dr. John Coligan)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The amino acid requirements of a functional influenza virus signal peptide were investigated using the influenza hemagglutinin (HA) cDNA-SV40 expression system in African green monkey kidney (AGMK) cells. Local site-specific mutagenesis was carried out to generate a series of recombinants of HA-SV40 containing point mutations in the region of the influenza virus hemagglutinin (HA) gene that codes for the signal peptide sequences. These mutant HA-SV40 recombinants were used to transfect AGMK cells in order to achieve expression of mutant hemagglutinins. Functional characterization of such HA products by cell surface immunofluorescence assay, hemadsorption and analysis of glycosylation showed that a majority of the mutations had no effect on functional properties of HA. However, one isolate (mutant 28) that sustained several mutations including an amino acid substitution at the signal cleavage site was defective with regard to cell surface expression. Amino acid sequence analysis of the NH_2-terminus of mutant HA showed that the intracellularly accumulated HA failed to undergo signal cleavage. Also, the defective mutant HA contained only endoglycosidase H sensitive carbohydrate components that are added in the endoplasmic reticulum. These findings suggest that HA containing an uncleaved hydrophobic signal sequence translocates across the microsomal membrane but fails to proceed to the Golgi apparatus where endoglycosidase H resistant carbohydrates are incorporated. Point mutagenesis using a defined oligonucleotide primer has been attempted with the intention of isolating a specific cleavage mutant that will allow us to confirm that the signal cleavage defect present in mutant 28 is indeed responsible for its defect in HA translocation and cell surface expression.

TERMINATED 1987

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00366-05 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Biology of Dengue Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Co-PI:	Bangti Zhao, Ph.D.	Visiting Associate	LID, NIAID
	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
Others:	Robert M. Chanock	Chief	LID, NIAID

COOPERATING UNITS *(if any)* Dept. Virus Diseases, Walter Reed Army Inst. of Research, Wash., D.C. (Donald Burke and Kenneth Eckels)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	0.8	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Genetic manipulation of the viral genome at the molecular level has been an attractive approach to development of stable live attenuated vaccines for prevention of viral infection. Attempts were undertaken to produce: (a) full-length cloned dengue DNA or (b) its RNA transcript synthesized in vitro that is infectious after introduction into permissive mosquito or primate cells. Initially, seven overlapping DNA segments spanning the entire dengue type 4 virus genome were used to construct a complete DNA copy by joining DNA fragments cleaved by endonucleases at shared sites. The unit-length dengue DNA of 10,644 nucleotides appeared to be stable in pBR322 plasmid as a molecule as shown by restriction enzyme digestion. The following sequences were added to the dengue recombinant DNA in an attempt to increase infectivity of dengue nucleic acid: (1) an SV40 promoter to increase transcription of RNA from dengue DNA following its transfection into primate cells; (2) an SP-6 promoter to increase in vitro synthesis of RNA from a dengue DNA template. Under assay conditions which were efficient for detection of infectivity of dengue virion RNA, we failed to detect invectivity of either dengue DNA RNA transcripts in cell culture. Presence of a short length of non-dengue genomic sequences at the 5' and 3' ends of the complete dengue cDNA may be responsible for its failure to be infectious. Experiments are currently in progress to remove these additional non-viral nucleotides so that only precise dengue RNA sequences are transcribed in vivo or in vitro including a 7mG cap at the 5'-terminus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00407-03 LID
(Terminated 1987)

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Complementation of Influenza Mutants by Cloned Viral Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kevin Ryan, Ph.D.	Staff Fellow	LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
	Erich Mackow, Ph.D.	Staff Fellow	LID, NIAID
	Robert Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Efforts to achieve persistent expression of influenza A virus cloned DNA in cells permissive for virus infection were initiated because such cells should be useful for investigation of the molecular biology of influenza virus and for isolation of specific viral mutants through complementation by the expressed gene. In this manner, naturally occurring or laboratory engineered mutants containing viable deletion mutations could be isolated for evaluation of their level of attenuation. Initially, simian cells permissive for influenza A virus infection were stably transformed with a full length cloned influenza A nucleoprotein gene under the control of an inducible metallothionein promoter and linked to a dihydrofolate reductase gene to facilitate selection of transformed cells. The tranformed cells which were selected synthesized an influenza A viral nucleoprotei which was indistinguishable from the nucleoprotein synthesized in virus-infected cells with respect to molecular weight and intracellular localization. It was estimated that transformed (CV1-NP) cells produced only 1% of the amount of nucleoprotein synthesized in simian cells infected with influenza A virus. Nonetheless, when these cells were infected with influenza virus mutants which synthesized temperature sensitive nucleoprotein, protein expressed by the cloned gene was able to complement the synthesis of plus-strand and minus-strand viral RNA for some mutants and only plus-strand synthesis for other mutants. This indicated that the nucleoprotein expressed in the transformed cells from cloned influenza A virus nucleoprotein cDNA exhibited functional activity. Furthermore, under appropriate conditions CV1-NP cells complemented the replication of ts mutant viruses. This complementation effect is currently being analysed to gain a better understanding of the biological functions of the viral nucleoprotein and to develop a strategy for isolation of viable deletion mutants.

(TERMINATED 1987)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00408-03 LID
(Terminated 1987)

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Attempts at Allele Replacement of NA Gene of Influenza A Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Lewis J. Markoff, M.D. Medical Officer LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	0.9	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The genome of many plus-strand RNA viruses is infectious. In contrast, the genome of negative-strand or of double-stranded RNA viruses is not infectious. We are attempting to render a single gene segment of a negative strand RNA virus, influenza A virus, infectious by rescuing RNA copies of the gene prepared in vitro from cloned full-length DNA, in cells infected with an influenza A virus. To facilitate such a "rescue", we chose a gene that bears a host range mutation that has a selective advantage. A full-length ds DNA copy of the influenza A WSN virus NA RNA was cloned. A complete cDNA copy of the gene was then expressed in an SV40 vector as a fully functional NA glycoprotein. Having established that the NA DNA was functional, we subcloned the DNA into a plasmid vector containing promoter sequences for phage RNA polymerases. (+) strand copies of NA DNA were generated in vitro. These transcripts necessarily contain short sequences at 5' and 3' ends copied from vector DNA. In the rescue experiment, primary AGMK cells were infected with a suitable recipient influenza A virus strain. After infection, cells were transfected with (+) strand RNA transcripts at high multiplicity. After 16 to 18 hours, the medium and the AGMK cell monolayer were harvested and inoculated onto a MDBK cell monolayer. Growth in MDBK cells would indicate that "rescue" of the WSN NA gene had occurred. Thus far, this approach has not succeeded. We are re-engineering the transcription vector to produce discrete NA copies of WSN NA DNA that lack terminal non-influenza sequences. We also plan to repeat the experiment in continuous monkey cell lines that persistently produce the influenza nucleoprotein, in one case, or the three influenza RNA polymerases, in another case. Possible such transformed cell lines may facilitate the rescue of transfected RNA.

(TERMINATED 1987)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00458-02 LID INACTIVE 1987

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic Variations Among Dengue Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Yi-ming Zhang, M.D.	Visiting Fellow	LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The dengue virus subgroup of the flavivirus family is comprised of 4 distinct serotypes (type 1 to type 4). Intratypic variations have been detected within several serotypes by oligonucleotide fingerprint analysis and by virus neutralization. Dengue type 4 variants from the Caribbean recovered during 1981-1982 appear to be different from the dengue 4 prototype (H241) isolated in the Phillipines in 1956 as well as recent isolates from Southeast Asia. During the past two years, dengue type 4 was the most frequent cause of dengue hemorrhagic shock in Bangkok, in contrast to the earlier epidemic pattern in which dengue type 2 viruses were most often implicated. For these reasons it is important to define in molecular terms: (a) genetic stability and diversity of dengue 4 viruses isolated over a 30 year interval and (b) the possible involvement of specific virus strains, i.e., "virulent viruses", in severe dengue disease. Two dengue type 4 variants, the prototype strain H241 and strain 2123 isolated from a hemorrhagic shock patient, were chosen for cloning and DNA sequence analysis. Several specific oligonucleotide primers corresponding to the established dengue 4 sequence (Dominica strain) were tested for their ability to prime reverse-transcription. At least one oligonucleotide effectively primed each dengue genomic RNA and yielded cDNA 5,000 nucleotides in length. These cDNA products will be cloned by the procedure established earlier. Cloned DNA will then be analyzed to determine the sequence coding for the two protective antigens, the envelope glycoprotein and the NS1 nonstructural protein. Finally, sequences will be compared in an attempt to identify hypervariable regions in the E and NS1 glycoproteins.

INACTIVE 1987

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00459-03 LID

PERIOD COVERED

October 1, 1986 to September 31, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Expression of Dengue Virus Proteins Using Baculovirus as a Vector

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Yi-Ming Zhang, M.D.	Visiting Fellow	LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID

COOPERATING UNITS *(if any)*

Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research (K. Eckels)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The baculovirus expression system was used for the synthesis of dengue viral proteins from cloned DNA sequences. A stable recombinant baculovirus containing the Bgl II cleaved DNA fragment coding for the three structural proteins [capsid (C), matrix (M) and envelope (E)] and nonstructural proteins NS_1 and NS_{2A} was constructed. Recombinant virus infected insect sf9 cells exhibited positive immunofluorescence staining with antibodies specific for dengue E, Pre M (the precursor of M) or NS_1 glycoprotein. This indicates that the dengue DNA sequence was transcribed and the resulting mRNA was properly translated in insect cells to produce these dengue glycoprotein products. Analysis of proteins produced in baculovirus recombinant infected cells by SDS-polyacrylamide gel electrophoresis followed by "western" blotting identified E and NS_1 (and probably Pre M) similar in size to authentic proteins produced during dengue virus infection. These observations suggested that proteolytic cleavage and glycosylation of these dengue proteins was normal in the baculovirus expression system. Immunization of rabbits with lysates from baculovirus recombinant infected sf9 cells induced antibodies specific to each of the three dengue glycoproteins as indicated by radio-immune precipitation. A protection study in mice is currently in progress to determine whether immunized animals are resistant to challenge by dengue virus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00475-02 LID

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Nucleotide Sequence of Genes for Dengue Virus Type 4 Nonstructural Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Zhao, Ph.D.	Visiting Associate	LID, NIAID
Others:	Y. Makino, Ph.D.	Visiting Associate	LID, NIAID
	C.-J. Lai, Ph.D.	Head, MVB Section	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.3	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The single strand viral RNA genome of dengue type 4 virus has been completely sequenced. The genome contains 10,644 nucleotides with a single long open reading frame that encodes a polyprotein of 3386 amino acids. The polyprotein is cleaved post-translationally into the full complement of viral proteins by a mechanism similar to that proposed for gene expression of other flaviviruses. Homology alignment of the dengue type 4 virus polyprotein with the polyproteins of two other flaviviruses, yellow fever and West Nile viruses, indicated that the 5' end of the dengue viral RNA encodes three structural proteins designated capsid (C), matrix (M) and envelope (E). The 3' terminal 8200 nucleotides encode seven nonstructural (NS) proteins designated NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. NS1 is the major NS glycoprotein produced during viral infection, while NS3 and NS5 contain polymerase-like amino acid sequences. Except for NS1, the function of dengue NS proteins is not known. Dengue NS proteins are more highly related to West Nile (WN) or Murray Valley encephalitis (MVE) virus NS proteins than to yellow fever (YF) virus NS proteins. In the region of the polyprotein containing the nonstructural proteins there is considerable conservation of hydrophobicity and a consensus sequence prevails at many proteolytic cleavage sites implying a close functional relationship for corresponding NS proteins. In view of the recent finding that the YF NS1, as well as dengue NS1, induces resistance in mice to challenge with the homologous virus, it is likely that the dengue NS1 protein may prove useful in immunoprophylaxis against dengue infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00476-02 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Engineering the Genome of Dengue Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
Others:	Michael Bray, Ph.D.	IRTA, MVB Section	LID, NIAID
	Robert M. Chanock	Chief, LID	LID, NIAID

COOPERATING UNITS *(if any)*
WRAIR, Div. of Communicable Diseases and Immunology, WRAIR, Washington, D.C. (P. Summers and K. Echols)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previous results showed that cells infected with recombinant vaccinia virus containing cloned dengue DNA sequences produced what appeared to be authentic dengue Pre M, E, and NS1 glycoproteins. However, most animals inoculated with this recombinant virus failed to develop immune responses. In order to improve the immunogenicity of recombinant DNA produced dengue proteins, experiments were initiated to produce individual dengue antigens which were expressed on the cell surface or secreted extracellularly. The hemagglutinin (HA) of influenza virus was chosen to provide the N-terminal signal as well as the C-terminal anchor sequences that are required for intracellular protein transport. DNA sequences coding for the E glycoprotein were constructed by joining the Sal I/Acc I cleaved dengue DNA fragment to chemically synthesized oligomers. To prepare expression vector DNA, internal sequences of HA were removed by XbaI and XhoI digest and subsequently by Bal 31 for further extension from the cleavage site. This collection of HA deletions will be used for insertion of the E DNA sequences and expression of the recombinant DNA using the SV40 system. Recombinant SV40 virus producing the chimeric HA-E protein on the cell surface or secreted extracellularly will be identified by immunofluorescence assay. Finally, the DNA sequences expressing the chimeric protein will be isolated and used for construction of live recombinant vaccinia virus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00477-02 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression of Dengue Viral Genes in Vaccinia Recombinant Virus.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Bangti Zhao, Ph.D.	Visiting Associate	LID, NIAID
	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
Others:	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(if any)*
Laboratory of Viral Diseases, NIAID (B. Moss); Walter Reed Army Institute of Research (P. Summers & K. Eckels)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The initial goal in this project was to express both protective antigens of dengue virus, i.e., the E glycoprotein and the NS$_1$ nonstructural glycoprotein from cloned dengue cDNA using vaccinia virus as a vector. A recombinant vaccinia virus containing cloned DNA sequences coding for the three structural proteins and nonstructural proteins NS$_1$ and NS$_{2a}$ of dengue type 4 virus was constructed. Infection of CV-1 cells with this recombinant virus produced dengue structural proteins as well as the nonstructural protein NS$_1$. These proteins were precipitated by specific antisera and exhibited the same molecular size and glycosylation patterns as authentic dengue viral proteins. Infection of cotton rats with the recombinant virus induced NS$_1$ antibodies in one of 11 animals. However, an immune response to either the pre M or the E glycoproteins was not detected. A low level of expression of dengue genes by the vaccinia virus recombinant was probably the explanation for the limited serologic response to these dengue antigens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00499-01 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression of Selected Dengue E Protein Sequences by Recombinant Vaccinia Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael Bray, M.D.	IRTA Fellow	LID, NIAID
Others:	Lewis Markoff, M.D.	Medical Officer	LID, NIAID
	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(if any)*
Laboratory of Viral Diseases, NIAID, (B. Moss)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recombinant DNA techniques were employed to construct chimeric genes encoding a truncated portion of the respiratory syncytial virus (RSV) G glycoprotein ligated in-frame to a nearly complete dengue type 4 envelope (E) glycoprotein gene or to a smaller portion of the E gene. These genes were inserted into vaccinia virus, under control of the P7.5 promoter, to obtain expression of the chimeric proteins in infected cells. Previously, the RSV G glycoprotein gene was successfully expressed in vaccinia virus; the glycoprotein was transported to the cell surface, and infected animals developed a protective immune response. In contrast, a previous vaccinia virus recombinant which expressed dengue structural protein genes synthesized proteins similar to those produced during dengue virus infection, but cell-surface expression could not be demonstrated, nor was an immune response to structural proteins observed in most vaccinated animals. The strategy of the present project is to use processing signals in the amino terminal sequence of RSV G to bring about transport of a fusion protein containing dengue virus envelope E protein sequences to the cell surface. Truncated RSV G sequences were ligated to a fragment of the dengue E gene coding for 380 amino acids which represent about 80% of the length of this glycoprotein. Chimeric proteins expressed by vaccinia virus were recognized by polyvalent dengue virus antiserum and by monoclonal antibodies to E, but cell-surface localization could not be demonstrated, and immunization of cotton rats failed to induce an antibody response. Recombinants coding for subfragments of E, ranging from 28 to 70% of the protein, have been constructed and will be tested in the same manner. This approach may aid in localizing epitopes recognized by monoclonal antibodies, and if immunization using subfragments is successful, it will be possible to determine antigenic sites which induce a neutralizing antibody response.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00500-01 LID

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunogenicity of Dengue Type 4 Virus Nonstructural Protein NS1

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Barry Falgout, Ph.D.	IRTA, MVB Section	LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
	Robert M. Chanock, M.D.	Chief, LID	

COOPERATING UNITS *(if any)* Scripps Institute, La Jolla, CA (Lerner); Walter Reed Army Institute of Research, Washington, D.C. (Eckels)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	0.8	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recent studies indicate that the nonstructural protein 1 (NS1) of dengue virus induces protective immunity in mice. Use of this dengue protein for immunization against infection would be particularly attractive, because protection would not be mediated by virus neutralization and hence immune enhancement could be avoided. Two approaches are being undertaken to elucidate the immunogenicity of the dengue type 4 NS1. First, a series of overlapping oligopeptides that span the entire NS1 amino acid sequence are being synthesized. These peptides will be used for mapping epitopes that are responsible for complement-fixing, binding of specific antibodies, and protection against virus infection. The second approach involves construction of recombinant vaccinia viruses for expression of the entire NS1, or various subsets of NS1 fragments, on the cell surface. A DNA clone coding for the entire NS1 and two DNA subclones have been produced. One subclone codes for the first 267 amino acids of NS1 and the other encodes amino acids 267 to 383. Construction of recombinant vaccinia viruses containing these NS1 sequences are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00501-01 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of the Processing of Dengue Viral Glycoproteins.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Lewis Markoff, M.D. Medical Officer LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mechanism for selective translocation of flavivirus proteins across the ER for glycosylation and probably for virus assembly is not understood. Others in our laboratory cloned and sequenced DNA representing the full-length of the dengue virus type 4 (strain 814669) genome. Expression in vaccinia of a 4kb segment of dengue DNA coding for the viral structural proteins C, pM, M, and E, and the major nonstructural (glyco)protein, NS1, suggested that in the absence of down-stream additional nonstructural gene products normal cleavage and glycosylation occurred. This observation suggested that cleavage at the C-pM, pM-M, M-E, and E-NS1 sites in the polyprotein is catalyzed by a cellular protease or that one of the expressed dengue proteins is itself a protease, but they do not shed light on translocation.

We are testing the hypothesis that an N-terminal candidate signal sequence in pM is necessary to initiate the translocation of pM/M, E, and NS1. Cleavage would be catalyzed by the signal cleavage enzyme within the ER and occur pari passu with translocation. A smaller 1 kb dengue virus DNA segment encoding C, pM/M, and the M-E cleavage site has been cloned into an SV40 shuttle vector, pSV2330, previously described. Mutations in the DNA coding for the putative signal in pM that delete the signal or alter it by replacing hydrophobic amino acids with hydrophilic ones have been generated by site-directed mutagenesis. Expression of C and pM/M in monkey cells will reveal whether there is a requirement of E or NS1 for normal cleavage, as well as whether there is a signal function at the N-terminal hydrophobic region in pM. If C and pM/M are normally processed from the wild type DNA, and if the signal in pM is confirmed by phenotype of pM mutants, the 4 kb dengue DNA fragment encoding C, pM/M, E, and NS1 will be reconstructed using pM signalless mutant DNA. The polar effect of the functional signal deletion on the expression of E and NS1 will then be determined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00502-01 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Antigenic Analysis of the Dengue Envelope Glycoprotein Using Synthetic Peptides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lewis J. Markoff, M.D.	Medical Officer	LID, NIAID
Others:	Salome Kruger	Bio. Lab. Aid	LID, NIAID
	Michael Bray, M.D.	IRTA	LID, NIAID
	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(if any)*

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Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	0.8	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The antigenic structure of the envelope (E) glycoprotein of dengue virus was studied by analyzing the antigenicity of 38 overlapping "nested" peptides (synthesized by our collaborators at Scripps Research Institute of the Scripps Clinic) which span the predicted amino acid sequence of the E of dengue virus type 4, strain 814669. The genome of this strain was previously cloned and sequenced in our laboratory. ELISA was used to detect antibody binding. Eight distinct linear epitopes were identified. Two peptides react strongly with polyclonal hyperimmune mouse ascitic fluid directed against strain 814669 (O.D. max.≥1.2; titer ≥1:1,000) and 4 peptides clustered at the NH_2-terminus of the molecule react weakly with the same ascitic fluid (O.D. max 0.5-0.6; titer ≥1:80). Two additional peptides bind 2 monoclonal antibodies (mabs) directed against a closely related dengue 4 strain (H241). One of these mabs neutralizes strain H241. Currently, antipeptide antibodies are being prepared in rabbits. Preliminary results indicate that antiserum to one peptide (73B), that strongly binds polyclonal anti-814669 ascitic fluid, immune precipitates E and neutralizes strain 814669 at a titer of 1:10 in a plaque reduction assay. Protection studies in mice using peptide 73B as an immunogen and expression of the sequence coding for 73B at the surface of vector-infected cells are planned. In a related study of NS1, the nonstructural glycoprotein of dengue virus which is thought to mediate antibody recognition of infected cells, we analyzed an additional 13 peptides which are scattered throughout the linear sequence of the dengue type 4 virus NS1. One of these peptides is strongly reactive with polyclonal hyperimmune 814669 mouse ascitic fluid in ELISA. Additional NS1 peptides are being synthesized so that a complete overlapping set will soon be available to finish our analysis of this protective protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00503-01 LID

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Expression of Dengue Virus Antigens Using A Synthetic Signal Peptide.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lewis Markoff, M.D.	Medical Officer	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

One of the major goals of our laboratory is the development of an effective live dengue viral vaccine. A vaccinia recombinant bearing a fragment of the dengue genome coding for all the structural proteins and the major nonstructural protein (NS1) of the virus failed to induce detectable dengue antibodies in experimental animals. It is possible that the lack of immunogenicity of this recombinant was related to failure of the protective dengue viral antigens (envelope, (E), and NS1) to be expressed at the surface of vector virus infected cells. The relationship between cell surface expression and immunogenicity of vaccinia recombinants may be inferred from work done in several other laboratories, as well as our own. Our difficulty in attaining cell surface expression for the dengue antigens is most likely related to the fact that E and the matrix protein (M) are not expressed at the cell surface during the normal course of dengue viral infection. Therefore, processing of E and M coded for by the vaccinia recombinant would not be expected to result in surface expression of the vector coded proteins The failure of RSV G-dengue E chimeras, including the large fragment of E, to be expressed at the cell surface does not necessarily indicate that this fragment bears signals or conformational constraints that interdict cell surface expression Instead, the problem may reside in a conformational interaction between the G and E sequences at the amino acid level that results in sequestration in or before the Golgi or the NH_2-terminus of G may determine an unusual pathway through the Golgi to the cell surface that can't be negotiated by the E sequences. To determine whether the large (Hae III) fragment of E included in the first three RSV G-dengue E constructs is able in and of itself to traverse the Golgi and reach the plasma membrane, an attempt has been initiated to express the Hae III fragment of E downstream from a cleaveable signal sequence in an SV40 expression vector. The SV40/HA signal/Hae III E recombinants will then be scored for secretion of the E fragment coded for by the Hae III genomic segment.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00333-06 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A Longitudinal Study of Viral Gastroenteritis in Infants and Young Children

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Albert Z. Kapikian, M.D.	Head, Epid. Sect.	LID, NIAID
Others:	Koki Taniguchi, Ph.D.	Visiting Associate	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
<0.1	<0.1	0.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rotaviruses have been studied extensively predominantly by cross-sectional approaches. Such studies have yielded essentially "numerator" data which indicated that rotaviruses are a major cause of diarrheal illness. There have been few longitudinal gastroenteritis studies yielding important epidemiologic information. Therefore we initiated an examination of anal swab and serum specimens obtained during a previous long-term longitudinal study (1955-1969) at Junior Village, a welfare institution for normal, homeless children. Anal swabs and blood specimens were obtained routinely. Surveillance was carried out by a trained medical staff. As reported previously, 139 rotavirus strains were detected with the characteristic seasonal distribution. It should be possible to establish the serotypic diversity of these strains. The subgroup pattern of tested strains was of special interest in that both subgroup 1 and subgroup 2 viruses were detected. In addition, as noted previously, sequential sera from 384 children in residence sometime between May 19, 1963-May 31, 1966 have been tested for CF antibody to the "O" agent. 150 (40%) of the children experienced at least one rotavirus infection; 11 had a second infection and one a third infection. For the period from May 22, 1966-May 21, 1969 65 (36%) of 182 children (some overlap with previous period) experienced at least one rotavirus infection, with 6 having a second infection.

With the recent development of a simple, conclusive serotyping ELISA by Dr. Taniguchi et al. in Japan, we are now embarking on a long awaited project of serotyping strains from this study to determine the distribution of the 4 epidemiologically important rotavirus serotypes over the 4 year period of the surveillance. Since our source for antigen is from anal swabs which characteristically contain small quantities of antigen, we are attempting to cultivate these strains in tissue culture for serotyping by ELISA. Thus far 25 of 35 anal swabs have yielded rotavirus antigen following first passage in MA104 cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00334-05 LID
TERMINATED 1987

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of Monoclonal Antibodies to Rotavirus Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Karen Midthun, M.D.	Medical Staff Fellow	LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20982

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to devise a practical, quick assay for serotyping human rotavirus isolates, repeated attempts at isolating monoclonal antibodies directed at the major neutralization protein of human rotaviruses have been made. In the past, monoclonal antibodies directed at the outer capsid proteins VP$_3$, the hemagglutinin, and VP$_7$, the major neutralization protein, of RRV were isolated. Screening by hemagglutination-inhibition assay was of key importance in identifying monoclones directed at the outer capsid proteins of RRV. In order to isolate monoclonal antibodies directed at the major neutralization proteins of human serotypes 1, 2, and 4, Balb/C mice were immunized with human rotavirus x RRV reassortants which had human serotype specificity but contained the 4th gene (the hemagglutinin VP$_3$) and also the remaining genes from RRV. The mice were immunized approximately 3-4 times over a period of 2-4 months with partially purified virus. The fusion ratio was 10 spleen cells per NS-1 myeloma cell.

Screening of several fusions by hemagglutination-inhibition identified some monoclones which inhibited hemagglutination of the reassortant rotavirus and all of these monoclones were directed at the RRV 4th gene product, VP$_3$; none were directed at the human rotavirus VP$_7$ protein. A screening test involving neutralization of virus in 96 well tissue culture plates has been developed and will be used to screen for neutralizing antibodies to the different serotypes.

In addition, attempts are being made to develop an ELISA test which would enable serotyping of serotype 1 and 3 viruses by using monoclonal antibodies directed at the major neutralization protein of Wa (kindly supplied by Dr. Harry Greenberg) and at the major neutralization protein of RRV (which had been isolated in the past).

TERMINATED 1987

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00335-06 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Rotavirus Reassortants: Genetics and Use in Rotavirus Vaccines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Karen Midthun, M.D.	Senior Staff Fellow	LID, NIAID
Others:	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
	Albert Z. Kapikian, M.D.	Head, Epidemiology Sec.	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID
	Koki, Taniguchi, Ph.D.	Visiting Associate	LID, NIAID

COOPERATING UNITS *(if any)*
Flow Laboratories, Inc. (L. Potash)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.50	0.25	0.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The rotavirus VP7 neutralization antigens of serotypes 1,2,3 and 4 were analyzed for genetic relatedness by hybridization of their genes using single human rotavirus gene substitution reassortants. Double-stranded (ds) genomic RNAs extracted from tissue culture adapted human rotaviruses (serotypes 1-4) were hybridized to single-stranded (ss) mRNA probes derived from human-bovine rotavirus reassortants representing serotype 1,2,3 or 4 which contained only the VP7 gene of their human rotavirus parent. When stringent conditions were used, bovine rotavirus genes did not hybridize to the corresponding genes of human rotaviruses and thus hybridization occurred only between the human rotavirus VP7 gene in the labeled probe and a related VP7 gene present in the human rotavirus under study. A high degree of homology was demonstrated between the probe and the VP7 gene of strains belonging to the same serotype, whereas hybrid bands did not form with VP7 genes from human rotaviruses belonging to a different serotype. This technique was also used to examine RNA extracted from stools of 15 children hospitalized with rotavirus diarrhea. All five viruses with a "short" RNA pattern shared homology with the DS-1 strain (serotype 2) VP7 gene; two of them had been previously adapted to tissue culture and shown to be serotype 2 strains by neutralization. Of the remaining 10 viruses with "long" RNA patterns, two hybridized to the D strain (serotype 1) VP7 gene, six hybridized to the P strain (serotype 3) VP7 gene, and two hybridized to the ST3 strain (serotype 4) VP7 gene. The stool specimens from 14 of these 15 children were also tested by ELISA with VP7 serotype-specific monoclonal antibodies directed at human serotype 1,2,3, or 4. Ten specimens were successfully serotyped with this method and there was complete concordance with the hybridization assay. It appears that serotype identification by hybridization with the reassortant probes is both sensitive and specific and circumvents the need for tissue culture adaptation of human rotaviruses since the genomic RNAs are purified directly from stool specimens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00338-06 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Cloning of Rotavirus Genes.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
Others:	Kim Green, Ph.D.	Staff Fellow	LID, NIAID
	Johnna Sears	Biologist	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.25	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have obtained and analyzed cDNA clones from several genes of various rotavirus strains. Single or double stranded RNAs were used for cDNA synthesis by reverse transcriptase after either polyadenylation and oligo (dT) priming or by direct priming with synthetic oligonucleotides corresponding to the known end sequences of the genes. Double stranded cDNA molecules were inserted into plasmid vectors by either dC./dG. tailing or direct blunt end ligation. In many cases the RNA templates used for reverse transcription consisted of individually isolated genes (by preparative PAGE); otherwise identification of the gene segment present in each clone was established by Northern blot hybridization, colony hybridization with cDNA probes from clones of known origin, or direct sequencing.

A collection of cloned copies of all the eleven genes has been generated. It includes copies of the VP7 gene of the animal rotavirus strains NCDV and UK (bovine), OSU (porcine), and RRV (simian) and the human rotavirus strains Wa, Montgomery, DS-1, P, M37, ST3 and VA70. Also, VP3 gene clones from RRV, DS-1, Wa and NCDV have been obtained. Direct dideoxy sequencing of either mRNAs or cloned cDNAs has been performed for the VP7 gene of a number of strains by using sequence-specific oligonucleotide synthetic primers. In addition to the OSU and NCDV VP7 glycoprotein gene sequences, the sequence of the RRV VP7 cloned gene has been determined as well as some of the sequences from cloned VP7 and VP3 genes from the DS1 strain, and VP3 gene of the Wa strain.

LMSF
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LMI
LPVD
LMM
LPD
LVD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00339-06 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Isolation and Serotypic Characterization of Human and Animal Rotaviruses.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute effiliation)*

PI:	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epidemiology Sec.	LID, NIAID

COOPERATING UNITS *(if any)*
Osaka National Hospital (Dr. N. Ikegami); National Institute of Health, Japan (Dr. S. Masumo)

LAB/BRANCH
Laboratory of Infectious Diseases

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Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	0.5	0.7

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Human rotavirus strain F45, which was originally isolated from a diarrheic child in Japan, was found to belong to serotype 9 (prototype strain HRV WI-61) by plaque reduction neutralization (PRN) assay.

HRV F1 was shown to belong to serotype 1 by PRN assay, although it was not reactive with any of the serotype-specific VP7 monolclonal antibodies developed by Drs. Urasawa and Taniguchi.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00340-06 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Experimental Studies in Animals with Various Rotaviruses and Their Reassortants.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Yasutaka Hoshino, D.V.M.	Visiting Scientist, ES	LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epidemiology Sect.	LID, NIAID

COOPERATING UNITS *(if any)*
Ohio Agricultural Research and Development Center, Ohio State University, Wooster, Ohio (Dr. L.J. Saif).

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rotaviruses possess 2 outer capsid proteins, VP3 and VP7, which are independent neutralization antigens. This was established previously in LID during studies in which the VP3 and VP7 genes were segregated by gene reassortment and their products analyzed for capacity to induce neutralizing antibodies and to serve as a target for virus neutralization. Recently these outer capsid proteins were analyzed for their capacity to induce immunity to infection and disease. For this purpose a single VP7 gene substitution reassortant was produced by mating porcine rotaviruses OSU (serotype 3) and Gottfried (serotype 4). The resulting reassortant possessed the VP3 gene of one parent and the VP7 gene of the other parent. Newborn, colostrum deprived piglets (which lacked maternally-derived antibodies) were infected with OSU, Gottfried or the reassortant. The piglets developed a similar titer of antibodies for VP3 and VP7. Significantly, the neutralizing activity of antibodies directed at VP3 was similar to that of antibodies directed at VP7. Three weeks later the piglets were challenged with OSU or Gottfried. Piglets infected at birth with the reassortant exhibited significant resistance to challenge with either parental virus indicating that VP3 and VP7 each play an important, independent role in host immunity to rotavirus infection and disease. Also, it should be noted that a reassortant rotavirus bearing the VP3 of one serotype and the VP7 of another serotype induced resistance to both serotypes. This observation has implications for the development of an alternate strategy for immunization against human rotavirus disease.

LMSF
LPB
LMI
LPVD
LMM
LPD
LVD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00341-06 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluation of Experimental Rhesus Rotavirus Vaccine in Infants and Children

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Albert Z. Kapikian, M.D.	Head, Epidemiology Section	LID, NIAID
Others:	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
	Karen Midthun, M.D.	Senior Staff Fellow	LID, NIAID
	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
	Koki Taniguchi, Ph.D.	Visiting Associate	LID, NIAID
	Robert M. Chanock, M.D.	Chief, LID	LID, NIAID

COOPERATING UNITS *(if any)*
Flow Lab.; U.Md.; Johns Hop. U.; U. Rochester; Vanderbilt U.; Marshall U.; U. Tempere, Finland; U. Umea, Sweden; Nat. Inst. Dermatol., Venezuela; Johns Hopkins U.; King Edward Med. Coll., Pakistan; Agency Int. Dev.; CDC.

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Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.9	0.8	2.1

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The need for an effective rotavirus vaccine is now clear. The goal of such a vaccine is to prevent severe rotavirus diarrhea during the first 2 years of life when this disease is most serious. An animal rotavirus strain, rhesus rotavirus (RRV), is under intensive study in LID as a vaccine candidate. This simian rotavirus has not been recovered under natural conditions from man and is thus not a virus of the human heritage. Although the genes of RRV exhibit significant divergence in sequence from the corresponding genes of human rotaviruses, this simian rotavirus shares neutralization epitopes on VP7 with human rotavirus type 3 and is thus similar to the human rotavirus type 3 when tested by neutralization. Our Jennerian approach to immunization in which a vaccine strain is derived from a non- human host is now undergoing extensive clinical evaluation for safety and efficacy in various studies in the United States and abroad. Two studies with the RRV vaccine have now been completed, 4 others are undergoing analysis or are in progress and one is about to begin. Most promising are the results from a clinical trial in infants and young children in Venezuela in which vaccine efficacy was 100% for the most severe form of rotavirus diarrhea. In this study, the predominant infecting strain belonged to the same serotype as the vaccine virus. In two other studies in which the predominant infecting strain belonged to a different serotype than the vaccine virus, the vaccine proved to be ineffective. Thus, it appears that serotype specific immunity may be necessary to achieve satisfactory protection against rotavirus diarrhea. In response to this need we have pursued the development of a multivalent vaccine which would induce resistance to each of the human rotavirus serotypes. For this purpose RRV has been employed as a donor of attenuating genes that are transferred to reassortant rotaviruses which bear the major neutralization protein (VP7) of serotype 1, 2 or 4. These single gene substitution reassortants which contain 10 RRV genes and the gene for a human rotavirus VP7 are now being evaluated in phase I clinical trials. To date these reassortants appear to be as attenuated and immunogenic as RRV.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00342-06 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of Gastroenteritis Viruses by Electron Microscopy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Albert Z. Kapikian, M.D.	Head, Epid. Sect.	LID, NIAID

COOPERATING UNITS *(if any)*
LID/NIAID (J. Ticehurst, R. Purcell); LVD/NIAID (G. Kotwal)

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The electron microscope (EM) has been a mainstay for study of fastidious gastroenteritis viruses. Despite the development of 2nd and 3rd generation tests, EM remains an indispensable tool: (1) as the "supreme court" when newer tests yield variable results; (2) in the search for new agents of viral gastroenteritis, (3) for visualizing the site of attachment of antibody to the virion in antigen-antibody reactions; (4) for serologic studies; (5) for direct visualization and fine structure characterization of the morphology of virus particles; (6) for studying specimens derived from individuals with diseases of unknown etiology (such as non-A, non-B hepatitis) by immune electron microscopy; and (7) for rapid diagnosis experimental and natural rotavirus infections by direct examination of stool preparations. As noted previously, two new techniques which may assume importance in our EM studies have been introduced into our laboratory. These include (1) solid phase immune electron microscopy (SPIEM) which has been described as being more sensitive than conventional IEM for detecting fastidious agents and (2) colloidal gold conjugated to protein A as a means of facilitating the recognition of the specific site of antigen-antibody interaction. Finally, since over 50% of the episodes of pediatric diarrhea are without known etiology, EM should continue to prove to be a valuable tool in the search for such agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00343-06 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Role of Norwalk-Like 27nm Virus Particles in Viral Gastroenteritis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Karen Midthun, M.D.	Senior Staff Fellow	LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS *(if any)*

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Laboratory of Infectious Diseases

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Epidemiology Section

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NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.3	0.3

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☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Marin County agent is a 27nm virus-like particle which was associated with two separate outbreaks of nonbacterial gastroenteritis in northern California in 1978 by L. Oshiro. The agent is morphologically similar to, but serologically distinct from the Norwalk, Hawaii and Snow Mountain agents as assessed by immune electron microscopy (IEM) or solid phase radioimmunoassay (RIA) antibody blocking assay. One ml of a safety tested, bacteria-free filtrate prepared from a stool specimen from one of the individuals ill during the original Marin County outbreak was administered orally to seventeen adult volunteers. None of these individuals developed definite clinical illness. Two additional volunteers later received a 20 ml inoculum in order to enhance the probability of inducing illness by increasing the dose. One of these volunteers developed a gastrointestinal illness characterized by nausea, vomiting, diarrhea and malaise. Interestingly, this illness started five days after administration of the fecal filtrate and lasted 36-48 hours. Examination by IEM of several diarrheal stool specimens from this volunteer demonstrated a large number of 27nm particles. These particles were shown to be identical to the Marin County agent in IEM studies using acute and convalescent sera from the original outbreak. A preliminary survey of a series of gastroenteritis outbreaks by use of RIA failed to implicate the Marin County agent as an important cause of epidemic gastroenteritis. Recently, a biotin-avidin immunoassay has been developed for detection of antibody to the Marin County agent. It appears equal in sensitivity to the RIA and is being used to screen further outbreaks of gastroenteritis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00346-06 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic Characterization of Rotavirus by Sequencing and Hybridization Techniques

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
Others:	Kim Green, Ph.D.	Staff Fellow	LID, NIAID
	Karen Midthun, M.D.	Senior Staff Fellow	LID, NIAID
	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
	Mario Gorziglia, Ph.D.	Visiting Associate	LID, NIAID

COOPERATING UNITS *(if any)*

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NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rotaviruses appear to undergo genetic variation by genetic drift or genetic shift (gene reassortment). Sequence analysis of rotavirus strains recovered over several years from a nursery in which asymptomatic infection occurred continuously suggests that the rotavirus genome does not undergo spontaneous mutation with high frequency. Previously when RNAs of rotaviruses isolated from asymptomatic newborn infants ("nursery strains") were compared by RNA-RNA hybridization with strains from ill infants, a marked difference in the sequence of the fourth gene was detected between the two groups of rotaviruses. The fourth gene is highly conserved among nursery strains, but it differs significantly from the corresponding conserved gene of strains isolated from ill infants. The conserved fourth gene of the nursery strains probably plays a role in their attenuation. The molecular epidemiology of this phenomenon was further analyzed using a dot/blot hybridization procedure which discriminates between of the fourth gene of virulent and asymptomatic strains. We analyzed additional rotavirus strains recovered from a hospital newborn nursery in which nosocomial transmission of asymptomatic rotavirus infection had persisted for several years or more. Each of the 11 strains analyzed possessed the conserved fourth gene associated with asymptomatic infection of newborns. However, several other strains recovered from sporadic asymptomatic newborn infections possessed a "virulent conserved" fourth gene. These infections did not occur in a nursery in which persistent transmission of asymptomatic infection had been documented. Hence, the association of a conserved fourth rotavirus gene sequence with persistent transmission of asymptomatic infection in the newborn nursery still holds. The sporadic occurrence of asymptomatic rotavirus infection in the newborn by a strain possessing the conserved sequence of virulent strains suggests that host factors also play an important role in expression of virulence.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00446-03 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Testing of Rotavirus Vaccine Candidates in Venezuela

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
Others:	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID

COOPERATING UNITS *(if any)*
Instituto de Biomedicina, Caracas, Venezuela (Dr. I. Perez-Schael, Dr. M. Vilar, Dr. N. Daoud, Dr. M. Perez)

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NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.4	0.5

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☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In phase I and II double-blind studies in Venezuela, the rhesus rotavirus (serotype 3) candidate vaccine was fed to over 140 infants (1-10 months old) and a similar number received placebo. Significant reactions were not observed. Overall, 69.3% of the vaccinees developed a seroresponse to rhesus rotavirus (RRV). During a similar study in newborn infants reactions were not observed after feeding a full dose (10^4 PFU) of vaccine. Although only 2 of 20 vaccinees developed a sero-response (as detected by neutralization assay) shedding of virus was observed in 19. Phase I studies have also been performed with two RRV reassortants representing serotypes 1 (D x RRV) and 2 (DS1 x RRV). In addition a group of 23 children received a bivalent preparation (RRV and D x RRV). Minor reactions (low grade fever 2, 3 or 4 days after vaccination) were seen in 10-25% of the children in these three groups as well as in a comparison group vaccinated with RRV alone. Shedding of virus was detected in over 67% of the vaccinees. Most of the children who received RRV and D x RRV shed both viruses in their stool. During a 1 year period of longitudinal follow-up of 247 children in the first RRV study, 5 episodes of rotavirus diarrhea were observed among the vaccinees versus 16 in the placebo group for an overall vaccine efficacy of 68%. Vaccine efficacy was 93% in infants who were 1-5 months old at the time of vaccination. The six most severe episodes occurred in the placebo group for a vaccine efficacy of 100% against severe diarrhea. The rotavirus strains recovered from ill infants or children were predominantly serotype 3 and thus the protection provided by the RRV vaccine was homotypic.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00450-03 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Sequence of the Fourth Rotavirus Gene and Its Role in Virulence

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mario Gorziglia, Ph.D.	Visiting Associate	LID, NIAID
Others:	Kim Green, Ph.D.	Staff Fellow	LID, NIAID
	Kazuo Nishikawa, M.D.	Visiting Fellow	LID, NIAID
	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
	Koki Taniguchi, Ph.D.	Visiting Associate	LID, NIAID
	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID
	Robert M. Chanock, M.D.	Chief, LID	LID, NIAID

COOPERATING UNITS *(if any)*

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NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.7	0.2

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The complete nucleotide sequence of the fourth gene of 8 human rotaviruses was determined by the dideoxy chain-termination method. Gene 4, which encodes VP3, is 2359 base pairs in length and contains a single long open reading frame of 2325 bases, capable of coding for a protein of 775 amino acids, with 5' and 3' noncoding regions of 9 and 25 nucleotides respectively. The VP3 of human rotaviruses contains identical N-terminal amino acid sequences, and there is conservation of arginine at the two trypsin cleavage sites as well as conservation of clusters of amino acids in different regions of the two VP3 cleavage products, VP8 and VP5. There are 14 regions in the VP3 protein sequence which exhibit the greatest variability. These regions may represent potential antigenic sites. Alignment of amino acid sequences of asymptomatic and virulent human rotavirus strains indicates a high degree of homology (96% or more) among the asymptomatic viruses (serotypes 1, 2, 3 and 4), while homology between asymptomatic strains and virulent viruses is considerably less (76%). A high degree of conservation of amino acid sequence (90-93%) is also observed among 3 of the virulent strains (serotypes 1, 3 and 4). At 89 positions in the protein sequence of VP3, an amino acid is conserved among asymptomatic rotaviruses, while a different amino acid is conserved among virulent rotaviruses. Notably, three of these differences are located within the short 6 amino acid cleavage region between VP8 and VP5. It is possible that some or all of this sequence dimorphism may be responsible for the difference in virulence of these two groups of human rotaviruses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00451-03 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunological Characterization of Rotavirus Antigens.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Yasutaka Hoshino, D.V.M.	Visiting Scientist, ES	LID, NIAID
Others:	Mario Gorziglia, Ph.D.	Visiting Associate, ES	LID, NIAID
	Kazuo Nishikawa, M.D.	Visiting Fellow, ES	LID, NIAID
	Kim Green, Ph.D.	Staff Fellow, ES	LID, NIAID
	Koki Taniguchi, Ph.D.	Visiting Associate, ES	LID, NIAID

COOPERATING UNITS *(if any)*
South Dakota State University (Dr. D. Benfield)

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INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	0.3	1.0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In an attempt to further delineate antigenic structure and function of rotavirus we have generated (i) a series of single gene substitution reassortant rotaviruses and (ii) a series of rotavirus mutants resistant to neutralizing VP7 monoclonal antibodies. Some of the reassortants are potential human rotavirus vaccine candidates. Serologic analysis of the escape mutants and sequence analysis of the VP7 gene of the mutants have revealed a complex structure of neutralization antigenic sites on the VP7.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00478-02 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of Adenovirus Expression Vectors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
Others:	Kim Y. Green, Ph.D.	Staff Fellow	LID, NIAID
	Johnna Sears	Biologist	LID, NIAID

COOPERATING UNITS *(if any)*

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Laboratory of Infectious Diseases

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INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

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- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The adenovirus genome has recently been engineered to allow the insertion of foreign genes in a manner similar to that used to construct vaccinia recombinants. Expression of the inserted foreign gene(s) during adenovirus infection may induce the formation of antibodies capable of neutralizing the virus from which the foreign gene was obtained.

We are testing this approach to develop recombinant rotavirus/adenovirus vaccines, hopefully more efficacious (more cross-reactive) and less reactogenic than the rhesus rotavirus vaccine. Current progress includes restriction mapping of the genome, cloning in plasmids of several restriction fragments corresponding to the E3 region and adjacent areas of the adenovirus 4 genome, and cloning of the NCDV rotavirus VP7 gene cDNA copy into a plasmid containing adenovirus 2 major late promoter cDNA. The right hand end of the adenovirus 4 genome (map units 83-100) has also been cloned. The VP7 glycoprotein gene of several human rotavirus strains and most of the fourth rotavirus gene from three different strains have been cloned in pBR322 or in the polylinker area of pUC vectors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00479-01 LID
(Merged with 00451-03
and Terminated)

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of Monoclonal Antibodies to Proteins of Rotavirus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Yasutaka Hoshino, D.V.M. Visiting Scientist LID, NIAID

COOPERATING UNITS *(if any)*

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Laboratory of Infectious Diseases

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Epidemiology Section

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NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.3	0.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An equine rotavirus strain FI-14 was shown to belong to serotype 3 by neutralization assay. In addition, it was found to react with both subgroup I and subgroup II monoclonal antibodies by ELISA, thus representing the first rotavirus strain to have both subgroup specificities. Using hybridoma technology, we successfully produced monoclonal antibodies directed against the major inner capsid protein VP6 (the sixth gene product) of FI-14 virus. These monoclonal antibodies reacted specifically with either subgroup I or subgroup II rotaviruses thus demonstrating that the VP6 of FI-14 virus has both subgroup I and subgroup II-specific epitopes. Four additional monoclones directed to the VP-6 of FI-14 demonstrated distinct reactivities by ELISA with a panel of 22 rotavirus strains derived from 11 different animal and avian species. Thus, at least six distinct epitopes were shown to exist on VP6 of FI-14 virus. When analyzed by radiommunoprecipitation, the molecular weight of the FI-14 virus VP6 (subgroups I and II) was found to be larger (approximately 45K) than the VP6 (approximately 42K) of rhesus rotavirus MMU18006 (subgroup I) or the VP6 of human rotavirus Wa (subgroup II). By RNA-RNA hybridization analysis, the FI-14 virus was shown not to share significant homology with any of the four known human rotavirus serotypes.

(MERGED WITH 00451-03 AND TERMINATED)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00504-01 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Sequence of the Sixth Rotavirus Gene

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mario Gorziglia, Ph.D.	Visiting Associate	LID, NIAID
Others:	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
	Albert Z. Kapikian, M.D.	Head, Epid. Sec.	LID, NIAID
	Robert M. Chanock, M.D.	Chief, LID	LID, NIAID

COOPERATING UNITS *(if any)*
BRB, NIAID (Dr. L. Maloy)

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.3	0.3

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The nucleotide sequence of the sixth gene that codes for the major inner capsid protein, VP6, of the human rotavirus strain 1076 (subgroup I), porcine rotavirus Gottfried (subgroup II), equine rotavirus strain H-2 (neither subgroup I nor II) and equine rotavirus strain FI-14 (both subgroup I and II) was determined. The sixth segment (+) strand RNA has a 5' and 3' nontranslated sequence of 23 and 142 nucleotides respectively, and encodes a VP6 protein of 399 amino acids. Alignment of amino acid sequences of the VP6 protein of strain FI-14 and subgroup II rotaviruses (Wa and Gottfried) indicated a high degree of homology (94%), while homology between strain FI-14 and subgroup I rotaviruses (SA-11, RF, and 1076) was somewhat less (90-92%). On the other hand a high degree of conservation of amino acid sequence (95-97%) was observed between the H-2 strain and subgroup I rotaviruses. Five regions that may contribute to subgroup epitopes were identified. Region A (amino acids 45, 56) and region C (amino acids 114, 120) may contribute to subgroup I epitopes and regions B (amino acids 83, 86, 89, 92), D (amino acids 312 or 314, 317 or 319) and E (amino acids 341 or 343, 350 or 352) may contribute to subgroup II epitopes. When analyzed by the Western blot technique monoclonal antibodies specific for VP6 epitopes shared by all rotaviruses were observed to react with both monomeric and trimeric forms of VP6, while monoclonal antibodies specific for a subgroup I or II epitope reacted only with the trimeric form of VP6. This observation and the sequence analyses suggest that subgroup antigenic specificity is determined by conformational epitopes produced by the folding of VP6 or the interaction between VP6 monomers.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00505-01 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Neutralization Epitopes on Rotavirus Outer Capsid Proteins.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mario Gorziglia, Ph.D.	Visiting Associate	LID, NIAID
Others:	Kazuo Nishikawa, M.D.	Visiting Fellow	LID, NIAID
	Koki Taniguchi, Ph.D.	Visiting Associate	LID, NIAID
	Kim Green, Ph.D.	Staff Fellow	LID, NIAID
	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID

COOPERATING UNITS *(if any)*
Gamble Institute of Medical Research, Cincinnati, Ohio (Ward)

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Laboratory of Infectious Diseases

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NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	0.6	0.5

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The VP7 and VP3 genes of seven antigenic mutants of simian rotavirus SA-11 (serotype 3) selected after 39 passages in the presence of SA-11 polyclonal antibodies were sequenced. These mutants were no longer neutralized efficiently by the polyclonal SA-11 antiserum used for their selection. These experiments were performed to determine the position and total repertoire of neutralization epitopes present on the two major surface proteins which constitute the protective antigens of simian rotavirus SA-11. Nucleotide sequence analysis of simian rotavirus SA-11 mutants selected by polyclonal neutralizing antibodies indicated that: a) most amino acid substitutions that occurred in these mutants were also observed in mutants selected with neutralizing monoclonal antibodies (N-mAbs) representing 4 distinct neutralization specificities, b) in addition, amino acid substitutions occurred that were not selected for by N-mabs, particularly in the V1 variable region of VP7, which appears to be a heretofore unrecognized immunodominant neutralization epitope, c) most neutralization epitopes appeared to be independent of each other because amino acid substitution in different sites did not affect the binding of specific N-mAbs to other sites, d) however, in a minority of instances neutralization epitopes on VP7 appeared to be interrelated in that amino acid substitution in one site affected binding of N-mAbs to another distant site, e) the repertoire of VP7 antigenic sites includes at least 4 neutralization sites which contain a total of 6 distinct neutralization epitopes, and f) the outer capsid protein VP3 contains at least two distinct neutralization epitopes.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00506-01 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Sequencing of Rotavirus Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kim Y. Green, Ph.D.	Staff Fellow	LID, NIAID
Others:	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
	Johnna Sears, M.S.	Biologist	LID, NIAID
	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Antigenic differences among rotaviruses detected by neutralization were investigated at the amino acid level by nucleotide sequence analysis of the gene coding for the major serotype-specific neutralization protein (VP7) of human rotavirus strains D, Montgomery, M37, DS1, HN126, Price, ST3, and VA70. The deduced amino acid sequences of the VP7 of these viruses were compared and six regions of sequence-divergence were identified on the VP7 of strains belonging to 4 different serotypes. These six regions of amino acid divergence were highly conserved within viruses of the same serotype. It is unlikely that only one or a few amino acids determine the serotype specificity of a rotavirus, instead a series of consensus sequences appear to specify each serotype. Regions of the VP7 gene encoding two such divergent regions were analyzed for twenty rotavirus field isolates of unknown serotype. Each of the 20 strains analyzed exhibited marked homology in these 2 regions to only one of the 4 human rotavirus serotypes. The serotype predicted by sequence analysis correlated well with the serotype subsequently identified by ELISA. Thus, it is possible to sequence the VP7 gene of a viral isolate and predict its serotype based upon homology to a consensus sequence for a known serotype. Although this would not be a practical method for most laboratories, it may prove useful in characterizing strains which cannot be identified by neutralization or monoclonal antibody ELISA. Sequence analysis of the gene coding for the VP3 protein of was completed for 2 serotype 1 human rotavirus strains, Wa (a virulent strain) and M37 (an asymptomatic strain).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00507-01 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Characterization of Two New Human Rotavirus Serotypes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kim Green, Ph.D.	Staff Fellow	LID, NIAID
Others:	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
	Karen Midthun, M.D.	Senior Staff Fellow	LID, NIAID
	Jorge Flores, M.D.	Visiting Scientist	LID, NIAID
	Albert Z. Kapikian, M.D.	Head, Epidemiology Section	LID, NIAID

COOPERATING UNITS *(if any)*

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NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Strain W161, isolated in the United States, and strain 69M, isolated in Indonesia, have each been described recently as a new human rotavirus serotype. The gene coding for the serotype-specific capsid protein VP7 was sequenced from both strains and compared to the known gene sequence of rotavirus serotypes 1, 2, 3 and 4. The sequence data, in correlation with plaque-reduction neutralization data, confirms that these two strains are unique.

Double-capsid particles of strain W161 were prepared and used to immunize BALB/c mice. Sera from the mice were positive for hemagglutination-inhibiting (HI) antibody specific for W161. Hybridomas secreting monoclonal antibodies specific for W161 will be prepared using these mice.

The gene coding for the capsid protein VP3 of W161 has been sequenced to within 200 bases from the 5' end. Preliminary analysis shows that the sequence of the amino acid cleavage region between VP8 and VP5 is similar to that of virulent rotaviruses.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00508-01 LID

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Naturally Occurring Rotavirus Reinfection.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

PI:	Karen Midthun, M.D.	Senior Staff Fellow	LID, NIAID
Others:	Koki Taniguchi, Ph.D.	Visiting Associate	LID, NIAID
	Albert Z. Kapikian, M.D.	Head, Epidemiology Sec.	LID, NIAID

COOPERATING UNITS *(if any)*

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NIAID, NIH, Bethesda, Maryland 20892

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0.6	0.3	0.3

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The development of an effective strategy for immunization against rotavirus gastroenteritis requires that we understand the extent and duration of immunity to virus infection and disease. Since there are four rotavirus serotypes that are important in causing human disease, as well as 2 newly identified serotypes of yet undetermined importance, both homotypic and heterotypic immunity must be examined. In order to obtain more information about the extent and duration of homotypic and heterotypic immunity, a study of children who experienced more than 1 episode of rotavirus diarrhea has been initiated. The rotavirus strains in 6 paired stool specimens from Egyptian children who have had 2 episodes of rotavirus gastroenteritis have been successfully serotyped by ELISA. In 3 cases, the same serotype caused reinfection; in the other 3 cases, a different serotype caused reinfection. Clinical and epidemiological information concerning these infections and re-infections are being obtained in order to interpret the significance of our observations.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00509-01 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Crossreactive Neutralizing Epitopes on Rotavirus Outer Capsid Protein VP7.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kazuo Nishikawa, M.D.	Visiting Fellow	LID, NIAID
Others:	Mario Gorziglia, Ph.D.	Visiting Associate	LID, NIAID
	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
	Koki Taniguchi, Ph.D.	Visiting Associate	LID, NIAID
	Albert Z. Kapikian, M.D.	Head, ES Section	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

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0.3	0.3	0.0

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☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In an attempt to identify interserotypic cross reactive neutralizing epitopes on VP7, several antigenic mutants resistant to cross reactive neutralizing monoclonal antibodies (nMabs) were selected and their VP7 gene was sequenced. Two N-mAbs were employed in this analysis; one (57-8) was produced against the Gottfried strain (serotype 4) and neutralized not only serotype 4 viruses but also serotype 3 and serotype 6 viruses, while the other N-mAb (22334) was raised against RRV (serotype 3) and neutralized both serotype 3 and serotype 4 (Gottfried) viruses.

The Gottfried neutralization escape mutant selected by mAb 57-8 had an amino acid substitution at position 94, whereas a ST3 (serotype 4) mutant selected with same N-mAb had a substitution at position 291. A Gottfried mutant selected with N-mAb 22334 had an amino acid substitution at position 213, whereas P (serotype 3) antigenic mutants had an amino acid substitution at position 96. These observations indicate that amino acid substitutions in these mutants selected with crossreactive N-mAbs, occurred in regions that show considerable variation between serotypes, i.e., variable region 2 (amino acids 87-96) and variable region 5 (amino acids 208-223) or low conservation region 2 (amino acids 291-309). Three possible explanations for these observations come to mind: (1) that the N-mAbs recognized different related epitopes when reacting with different strains or (2) that the epitopes which were analyzed are subject to allosteric effects from mutation at a distant site or (3) that the monoclonal binding site is located in a conformational site on VP7 that contains sequences that are widely separated on the linear map of this protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00510-01 LID

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Rotavirus VP7 Neutralization Epitopes of Serotype 3 Strains.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kazuo Nishikawa, M.D.	Visiting Fellow	LID, NIAID
Others:	Mario Gorziglia, Ph.D.	Visiting Associate	LID, NIAID
	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID
	Koki Taniguchi, Ph.D.	Visiting Associate	LID, NIAID
	Albert Z. Kapikian, M.D.	Head, Epid. Sec.	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

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☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The third serotype of human rotavirus shares neutralization specificity with viruses derived from monkeys, dogs, cats, horses, rabbits, and mice. In addition, viruses of this serotype include strains of all known subgroup specificities (i.e., I, II, neither I nor II, both I and II). The relationships of the major neutralization protein, VP7, of the various serotype 3 strains were analyzed by comparing nucleotide and amino acid sequence. This analysis was performed in an attempt to clarify antigenic relationships and to better understand the evolution of serotype 3 rotavirus strains which have established an extraordinarily broad host range.

Preliminary data from the deduced amino acid sequences suggest an overall homology of more than 85% in the VP7 protein; among the strains derived from the same animal species, an even higher degree of homology was observed. The amino acid sequence analysis of the rotavirus VP7 genes derived from a variety of rotaviruses of varying serotype indicated that there are six highly divergent, serotype-specific regions. These regions are divergent among viruses belonging to different serotypes but are conserved to a varying degree within a given serotype. Significant sequence conservation was observed among the various human and animal serotype 3 strains in variable region 2 (amino acids 87-101), but some sequence divergence was seen in variable region 5 (amino acids 208-223). Analysis of neutralization resistant mutants of simian rotavirus SA-11 selected by growth of virus in the presence of hyperimmune antiserum or neutralizing monoclonal antibodies indicated that dominant neutralization epitopes were located in variable regions 2 and 5. These findings suggest that at least two monoclonal antibodies should be used for serotype analysis, one directed against V2 and another directed against V5.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00511-01 LID

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Rotavirus Cross-Reactive and Serotype-Specific Neutralization Epitopes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Koki Taniguchi, Ph.D.	Visiting Associate	LID, NIAID
Others:	Mario Gorziglia, Ph.D.	Visiting Associate	LID, NIAID
	Kazuo Nishikawa, M.D.	Visiting Fellow	LID, NIAID
	Yasutaka Hoshino, Ph.D.	Visiting Scientist	LID, NIAID
	Albert Z. Kapikian, M.D.	Head, ES Section	LID, NIAID
	Robert M. Chanock, M.D.	Chief, LID	LID, NIAID

COOPERATING UNITS *(if any)*

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0.7	0.7	0.0

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☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Two outer capsid proteins of rotavirus, VP3 and VP7, bear neutralization epitopes. In tests employing VP3 and VP7 cross-reactive neutralizing monoclonal antibodies (N-mAbs) against human rotavirus (HRV) serotype 1, 3, or 4, we observed some intraserotypic antigenic variation as well as marked differences in reactivity patterns between serotype 2 strains and serotype 1, 3 and 4 strains. In contrast, nine other VP7 neutralizing monoclonal antibodies were serotype specific. Epitope analysis of antigenic variants of serotype 1, KU strain, selected by their resistance to N-mAbs showed that VP3 has at least 3, independent, cross-reactive neutralization epitopes, while VP7 has at least one cross-reactive epitope and at least five overlapping serotype-specific neutralization epitopes which contribute to a single antigenic site.

Nucleotide sequence analysis of the antigenic variants by primer extension permitted us to identify the nucleotide substitutions in the VP3 and VP7 genes of the antigenic variants. Amino acid changes were detected at residues 305, 392, 433 and 439 of VP3 and 94, 96, 97, 99, 211 and 213 of VP7. These data may prove useful in current efforts to develop vaccines against HRV infection.

LMSF

LPB

LMI

LPVD

LMM

LPD

LVD

LABORATORY OF MICROBIAL IMMUNITY
1987 Annual Report
Table of Contents

Z01 AI Project Number		Page
Summary		12-1
00131-20	Mechanism of Hypersensititvity In Inbred Histocompatible Guinea Pigs - Stone	12-4
00134-25	Control of Immunoglobulin Syntheses In Mice - Asofsky	12-5
00136-15	Characterization and Differentiation of Thymic Lymphocytes - Fowlkes	12-6
00141-11	Immune Responses to Malaria - Asofsky	12-7
00143-18	Genetic Control of the Antibody Response To Microbial Antigens - Baker	12-8
00144-23	Regulation of the Antibody Response To Microbial Polysaccharide Antigens - Baker	12-9
00145-20	Mode of Action of Thymus-Derived (T) Suppressor and Amplifier Cells - Baker	12-10
00146-13	Immunological Studies of Components Isolated From Bacterial Parasites, and Plants - Caldes	12-11
00153-09	In Vitro Responses of Human Peripheral Leukocytes - Chu	12-12
00423-04	Factor Mediated Regulation of B Cell Growth and Differentiation - Ennist	12-13

PHS-NIH
SUMMARY REPORT

ANNUAL REPORT OF THE LABORATORY OF MICROBIAL IMMUNITY, NIAID
October 1, 1986 to September 30, 1987

Richard Asofsky, M.D.
Chief, Laboratory of Microbial Immunity

Identification of a New Types of T-Cell

A unigue T cell receptor, designated TCR γδ has been found on a subset of CD4 8 thymocytes. The receptor can be precipitated from the cell membranes either with monoclonal antibody to CD3 or with antiserum to a γ peptide. The receptor consists of a disulfide-linked heterodimer with component polypeptides of 35 and 45 dK (reduced), noncovalently linked to the same CD3 components found on TCR αβ-bearing T cells. The TCR γδ-bearing cells can be propagated *in vitro* for short periods using a mixture of lymphokines. These cells are the first TCR-bearing cells to appear in thymic ontogeny. We believe that the cells are a distinct lineage of T cells, and that some mediate mature T cell effector functions.

A second group of thymocytes has been found as a subset of the CD4$^-$8$^-$ cells. It appears late in ontogeny, and unexpectedly expresses the products of a single TCR Vβ family of genes, Vβ8 (Dr. B.J. Fowlkes, LMI, NIAID; Drs. D. Pardoll, R. Schwartz, LI; Dr. A. Kruisbeek, NCI).

Investigation of Lineage Relationships Among Thymocytes

The predominant mature cortical type of thymocyte is CD4$^+$8$^+$ thymocytes intravenously, or of such cells intrathymically into irradiated congenic hosts failed to generate any CD8$^+$ of the donor type. Treatment of mice *in vivo* with antibody to CD8 had no effect on the development of CD4$^+$8$^-$ cells either in intact animals or in irradiated bone marrow chimeras. CD4$^+$8$^+$, TCR$^+$ cells appear late in thymic ontogeny (day 18) after the appearance of the first functional CD4$^+$8$^-$ and CD4$^-$8$^+$ cells. Preliminary work also suggests that isolated CD4$^+$8$^-$ cells may give rise to CD4$^+$8$^+$ cells. These data suggest that CD4$^+$8$^+$ cells are not the precursors of mature functional T cells and are instead a terminal lineage (Dr. B.J. Fowlkes, LMI, NIAID; Dr. A. Kruisbeek, NCI).

Separate Components Responsible for Mediating the Immunomodulatory Effects of mannan

Mannan extracted from *Candida albicans* is capable of both enhancing and suppressing the magnitude of the antibody response to various unrelated, non-cross reactive antigens. The effect produced depends upon the concentration of mannan given; high concentration enhance whereas low concentrations suppress the antibody response. The enhancing and suppressive effects of mannan could be reparated as to molecular size and change, indicating that mannan extracts contain distinct components with opposing immunomedulatory properties. (P.J. Baker and J. Domer, LMI, NIAID)

Genetic and Extra-genetic Influences in Susceptibility and Resistance to EAE

Clinical AE is elicitable using whole guinea pig spinal cord antigen in complete Freund's adjuvant in both female and male Strain 13 guinea pigs, but is induced with difficulty in Strain 2, except in a few older female animals. In reciprocal crosses to produce F_1 offspring, the female issue should normally be identical in degree of resistance to clinical EAE. However, an extra-genetic maternal influence was apparent, since female 13 x 2 (♀ is always mentioned first) were clearly more susceptible to clinical disease than female 2 x 13's. As in the parent strains, histological lesions of EAE appear in sensitized, but clinically healthy F_1 guinea pigs. Females of parent strains are always more susceptible than males but in the 13 x 2 cross there was a noticeable switch in sex-susceptibility (70% ♂ mortality vs 50% ♀ mortality). In 2 x 13's, there were no sex differences and a significant decrease in acute mortality. F_2 cross 13/2 x 13/2 showed a 20-25% acute mortality and no sex differences while F_2 2/13 x 2/13 manifested 34% female early mortality and 22% male deaths from acute EAE. Backcross 2/13 x 13 had 40% acute mortality. Backcross 13/2 x 2 was resistant. These results suggest a multiplicity of factors (genes) involving susceptibility and resistance to EAE (Dr. S. Stone and Ms. D. Amsbaugh).

Hybridomas Which Secrete Two Isotypes of Anti-SSS-III Antibodies

Immunization of mice with pneumococcal polysaccharide SSS-III together with allogeneic lymphoid cells produces a great enhancement of the anti-SSS-III antibody response with particular augmentation of the IgG response (all isotypes). At the peak of this response many individual secreting cells are found which make both IgM and IgG. Fusion of such cells with a non-producing mouse myeloma yielded several long-term cell lines secreting both IgM and IgG3 anti-SSS-III antibodies. Each line was a "double producer" by the following criteria: (a) many subclones of each, obtained by limiting dilution, produced IgM and IgG3; (2) such subclones, incorporated S_{35}-methionine into IgM and IgG3; (3) transplantation of subclones to mice resulted in the production of IgM and IgG3 paraproteins in mg. amounts; (4) individual cells stained with fluorochrome-labeled antibodies to both IgM and IgG3 (Drs. Chu, Baker, and Asofsky, Mr. Fauntleroy).

Autocrine Growth Factor Inhibits Induced Differentiation of a B Cell x B Lymphoma Somatic Cell Hybrid

TH 2.2 is a B cell x B lymphoma which is induced by LPS to partial terminal differentiation and IgM secretion. In limiting dilution analysis (LDA) uninduced cells clone with high efficiency, about 8 in 10. Most of these clones are macroscopically visible by 14 days' incubation (macroclones). LDA of induced cells shows a reduction in cloning efficiency to 5-6 in 10. Furthermore, only 2 in 10 are macroscopically visible; the remainder are microscopic clusters of 20-200 cells (microclones). Macroclones can be regrown in fresh medium; microclones will not grow further. Microclones show a high rate of IgM secretion, about 10 times higher than control, macroclones from uninduced cells or induced cells.

Conditioned medium from uninduced TH 2.2 cells contains a growth factor which blocks or reverses this terminal differentiation. Although the cloning efficiency is not improved (it is still 6 in 10) for induced cells, the proportion of macroclones is greatly increased, and IgM secretion in these clones is reduced. TH 2.2 thus synthesizes a growth factor which prevents the terminal commitment of the cells produced by inducing agents (Drs. Sulis, Asofsky, Ms. Brooks).

Functional Description of Two BCGF II-Like Factors

We have previously identified two BCGF II-like factors found in a human BCGF and murine D10 T cell sup, which can be distinguished by their differential reactivity in several murine BCGF II assays. Extending the characterization of these two factors, we showed that when they were simultaneously added to culture, they synergized to produce optimal IgM secretion by large murine B cells and murine BCL$_1$ B lymphoma cells. The same factors were tested in and IgM specific plaque assay and a similar synergistic response was observed for the large B cells, but not for the BCL$_1$ cells. Kinetic analysis of the time of action of the two BCF II-like lymphokines in the induction of the PFC response by large B cells indicated that human BCGF was required within the first 24 hours of a 4 day culture period, while D10 sup could be added as late as the final 15 hours without significant diminution of the response. These data provide further support for the existence of two distinct B cell stimulatory factors which cause growth and differentiation of activated B cells, and indicate that these two factors synergize to produce optimal Ig secretion (Drs. Ennist, Elkins and Howard).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00131-20 LMI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanism of Hypersensitivity in Inbred Histocompatible Guinea Pigs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	S.H. Stone	Research Microbiologist	LMI, NIAID
Other:	D.F. Amsbaugh	Biologist	LMI, NIAID
	T. Hamada	Visiting Fellow	LMI, NIAID
	S.J. Zigler*		
	C.S. Raine**		

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LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Experimental Pathology Section

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have been studying genetic differences in susceptibility to autoimmune encephalomyelitis (EAE) between Strain 13 (susceptible) and Strain 2 (resistant) guinea pigs. Strain 2 sensitized to guinea pig spinal cord antigen shows histological evidence of, but not clinical signs of EAE. In reciprocal crosses to produce F_1 offspring, the females (if not the males) should normally be identical in degree of resistance to EAE. Surprisingly, the 2 x 13 females are more resistant than 13x2 females, indicating there is a extragenetic maternal influence determining the outcome. The nature of this effect, and the differences between subpopulations of guinea pig T cells are being examined by cellular immunological approaches. The heterogeneity of T cell functions is being approached in mouse systems because of availability of suitable animals, and the current knowledge of their lymphokines.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00134-25 LMI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Control of Immunoglobulin Synthesis in Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Richard Asofsky	Chief	LMI, NIAID
Others:	Carol Sulis*		
	Teranishi Tsuyoski**		
	Hsiao-Kun Chu	Visiting Associate	LMI, NIAID
	Ada Brooks	Biological Lab Tech	LMI, NIAID
	Dov Pluznick	Visiting Scientist	NIDR, NIAID
	Mathias Dickel	Visiting Scientist	NIDR, NIAID
	Phillip J. Baker	Research Microbiologist	LMI, NIAID
	Michael Fauntleroy	Biologist	LMI, NIAID

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

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- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cells from mice immunized with SSS-III pneumococcal polysaccharide together with allogeneic lymphoid cells, a procedure which results in the transient appearance of cells which secrete both IgM and IgG3 anti-SSS-III antibodies were fused with myeloma line Sp 2/0. Hybridomas were isolated which secreted both IgM and IgG3 anti-SSS-III antibodies. These lines were "double producers" by the following criteria: (1) subclones were each double producers *in vitro*; (2) subclones transplanted into mice produced an IgM and IgG3 paraprotein each of which had antibody activity to SSS-III; (3) S-35 labeled antibodies of IgM and IgG3 types were produced by each subclone incubated *in vitro* with S-35 methionine; and (4) many individual cells stained with fluorochrome labeled antibodies to both IgM and IgG3.

LMSF
LPB
LPVD
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RMOR
LPD
LVD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00136-15 LMI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characterization and Differentiation of Thymic Lymphocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B.J. Fowlkes	Microbiologist	LMI, NIAID
Others:	D. Pardoll		LCMI, NIAID
	A. Kruisbeek		BRMP, NCI
	R. Schwartz		LCMI, NIAID
	J. Coligan		BRB, NIAID
	G. Stievenart		LCMI, NIAID
	H. Ton-That		LMI, NIAID
	D. Stephany		BRB, NIAID

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INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Evidence was obtained that cortical-type ($CD4^+8^+$) thymocytes are end stage cells, and not the precursors of other types of T-cell: (1) transfer of large numbers (10^8) of highly purified $CD4^+8^+$ thymocytes I.V. to irradicated congenic recipients; (2) intrathymic transfer of $CD4^+8^+$ thymocytes failed to generate any detectable donor-derived cells in the recipients; (3) treatment of mice in vivo with anti-CD8 antibodies had no effect on production of $CD4^+8^-$ cells; and (4) $CD3^+$, $CD4^+8^+$ thymocytes appear late in ontogeny (day 18) after $CD4^+8^-$, $CD4^-8^-$, and $CD4^-8^+$ cells. Finally, we have preliminary evidence that $CD4^+8^-$ cells may give rise to $CD4^+8^+$ cells.

We have identified a new type of T cell with a unique T cell receptor. The receptor, designated TCR $\gamma\delta$ is a heterodimer with disulfide-linked components of 35 and 45 kD, is noncovalently linked to the same CD3 components found in TCR $\alpha\beta$-bearing cells, and is precipitated either with monoclonal anti-CD3 or an antiserum to a γ peptide. These cells can be grown in vitro with a mixture of lymphokines. TCR $\gamma\delta$ cells are the earliest TCR-bearing cells in fetal ontogeny. We believe that some of these cells are functional and that they represent a distinct lineage from those bearing TCR $\alpha\beta$.

We have demonstrated the appearance late in thymic ontogeny of a group of cells which is $CD4^-8^-$ and in which a single TCR $V\beta$ family ($V\beta 8$) predominates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00141-11 LMI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immune Responses to Malaria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Richard Asofsky Chief LMI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

No work done this year in this project.

PHS 6040 (Rev. 1/84) GPO 914-918

LMSF
LPB
LPVD
LMM
RMOR
LPD
LVD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00143-18 LMI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT (*80 characters or less Title must fit on one line between the borders.*)
Genetic Control of the Antibody Response to Microbial Antigens

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	P. J. Baker	Head, MBIM Section	LMI, NIAID
Others:	M. Fauntleroy	Biologist	LMI, NIAID
	J. A. Rudbach*		
	B. Prescott**		

COOPERATING UNITS (*if any*)
*Ribi ImmunoChem Inc., Hamilton, MT 59840
**Biomedical Research Institute, Rockville, MD 20852

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Microbiology and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 0.8	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

C3H/HeN (responsive to Lipid-A) and C3H/HeJ (not responsive to Lipid A) were primed with sheep erythrocytes so as to generate significant immunological memory. When these mice were treated subsequently with bacterial lipopolysaccharide (LPS), significant polyclonal activation of memory cells was noted only in C3H/HeN mice. This suggest that the Lipid A moiety of the LPS molecule plays an important role in the activation of memory B cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00144-23 LMI

PERIOD COVERED
October 1, 1986 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of the Antibody Response to Microbial Polysaccharide Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. J. Baker	Head, MBIM Section	LMI, NIAID
Others:	K. Elkins	Staff Fellow	LMI, NIAID
	D. Ennist	Staff Fellow	LMI, NIAID
	J. Hiernaux	Visiting Scientist	LMI, NIAID
	P. W. Stashak	Microbiologist	LMI, NIAID
	M. Fauntleroy	Biologist	LMI, NIAID
	J. Domer*		
	B. Prescott**		
	J. A. Rudbach***		

COOPERATING UNITS *(if any)* *Department of Microbiology & Immunology, Tulane University, New Orleans, LA 70112; **Biomedical Research Institute, Rockville, MD 20852; ***Ribi ImmunoChem Inc., Hamilton, MT 59840

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Microbiology and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.2	0.7

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Although prior treatment with subimmunogenic amounts of some preparations of bacterial lipopolysaccharide (LPS) results in the development of immunological memory, prior exposure to others results in profound unresponsiveness or suppression. The results of preliminary experiments suggest that the later may be mediated by a subset of T cells possessing the Lyt2 cell-surface antigen.

Extracts of mannan derived from Candida albicans can both enhance and suppress antibody responses to unrelated antigen. These immunomodulatory effects can be separated on the basis of molecular size and charge, indicating that they are mediated by distinct components present in extracts of mannan.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00145-20 LMI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Mode of Action of Thymus-Derived (T) Suppressor and Amplifier Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. J. Baker	Head, MBIM Section	LMI, NIAID
Others:	K. Elkins	Staff Fellow	LMI, NIAID
	P. W. Stashak	Microbiologist	LMI, NIAID
	M. B. Fauntleroy	Biologist	LMI, NIAID
	B. Prescott*		

COOPERATING UNITS (if any)
*Biomedical Research Institute, Rockville, MD 20852

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Microbiology and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	0.8	0.6

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The ability of spleen cells adhering to Vicia Villosa lectin to augment the magnitude of the antibody response to Type III pneumococcal polysaccharide (SSS-III) was investigated. The reactive cells appear to be more characteristic of amplifier T cells, than the contrasuppressor T cells described by other investigators.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00146-13 LMI

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunological Studies of Components Isolated from Bacteria, Parasites, and Plants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G. Caldes	Chemist	LMI, NIAID
Others:	P. J. Baker	Head, MBIM Section	LMI, NIAID
	B. Prescott*		
	P. W. Stashak	Microbiologist	LMI, NIAID
	M. Fauntleroy	Biologist	LMI, NIAID

COOPERATING UNITS *(if any)*

*Biomedical Research Institute, Rockville, MD 20852

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Microbiology and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.5	0.6

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was discontinued as of October 30, 1985 with the retirement of Mr. G. Caldes, the Principal Investigator.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00153-09 LMI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (*80 characters or less Title must fit on one line between the borders.*)

In Vitro Responses of Human Peripheral Leukocytes

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	Hsiao-Kun Chu	Visiting Associate	LMI, NIAID
Other:	Richard Asofsky	Chief	LMI, NIAID

COOPERATING UNITS (*if any*)

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

  ☐ (a1) Minors

  ☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

No work done this year in this project.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00423-04 LMI

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Factor Mediated Regulation of B Cell Growth and Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Ennist	Staff Fellow	LMI, NIAID
Others:	R. Asofsky,	Chief,	LMI, NIAID
	P. Baker	Senior Scientist	LMI, NIAID
	K. Elkins	Staff Fellow	LMI, NIAID
	J. Miyazaki	Visiting Scientist	LDMI, NICHHD
	K. Ozato	Senior Scientist	LDMI, NICHHD
	R. Nordan	Staff Fellow	LG, DCBD, NCI

Others continued on next page

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have previously identified two BCGF II-like factors found in a human BCGF and murine D10T cell sup, which can be distinguished by their differential reactivity in several murine BCGF II assays. Extending the characterization of these two factors, we showed that when they were simultaneously added to culture, they synergized to produce optimal IgM secretion by large murine B cells and murine BCL_1 B lymphoma cells. The same factors were tested in an IgM specific plaque assay and a similar synergistic response was observed for the large B cells, but not for the BCL_1 cells. Kinetic analysis of the time of action of the two BCF II-like lymphokines in the induction of the PFC response by large B cells indicated that human BCGF was required within the first 24 hours of a 4 day culture period, while D10 sup could be added as late as the final 15 hours without significant diminution of the response. These data provide further support for the existence of two distinct B cell stimulatory factors which cause growth and differentiation of activated B cells, and indicate that these two factors synergize to produce optimal Ig secretion.

Other Professional Personnel Continued:

M. Howard, DNAX Research Institute, Palo, Alto, CA
K. Brechling, Duke University, Durham, NC
T. Hirano, Osaka University, Osaka, Japan
T. Kishimoto, Osaka University, Osaka, Japan
T. Teranishi, Osaka-Minami National Hospital, Kawachinago City, Japan

LMSF

LPB

LPVD

LMM

RMOB

LPD

LVD

LABORATORY OF MOLECULAR MICROBIOLOGY
1987 Annual Reports
Table of Contents

Z01 AI Project Number	Title	Page
Summary		13-1
00027-20	Basic Studies of Mycoplasmas - Tully	13-12
00190-09	The Molecular Genetics of Eukaryotic Cells and Their Viruses - Martin	13-13
00218-06	Biochemical and Chemical Studies on Retroviral DNA - Repaske	13-14
00219-06	Molecular and Genetic Analysis of Streptococci - LeBlanc	13-15
00222-06	Characterization of Endogenous Ecotropic and Xenotropic Murine Leukemia Virus - Theodore	13-16
00281-06	Molecular Cloning of Recombinant MuLV Proviral Sequences in Inbred Mice - Buckler	13-17
00300-06	Control of Resistance to Viral Leukemogenesis in Wild Mouse Population - Kozak	13-18
00301-06	Cellular Genes Involved in Transformation and MuLV Transmission in Inbred Mice - Kozak	13-19
00304-06	Pathogenesis of Disease Induced by Friend MuLV - Silver	13-20
00353-05	Structural and Functional Analysis of Endogenous Proviruses of Mice - Khan	13-21
00388-04	Functional Analysis of Human Retroviral Genes - Rabson	13-22
00395-04	Genetic and Molecular Analysis of Anaerobic Bacteria Indigenous to Humans - Smith	13-23

Z01 AI Project Number	Title	Page
00415-04	The Molecular Biology of Retroviruses Associated with AIDS - Martin	13-24
00433-03	Use of Retroviruses as Tagged Insertional Mutagens - Silver	13-25
00434-03	Studies on the Dissemination of Streptococcal Antibiotic Resistance Determinants - LeBlanc	13-26
00437-03	The Biology and Genetics of the AIDS Retrovirus - Hoggan	13-27
00438-03	The Molecular Biology of Cellular Oncogenes - Santos	13-28
00464-02	Plasmid Replication and Expression in Streptococci - Ranhand (This project has been terminated)	13-29
00466-02	Transcriptional Complexity of AIDS Retrovirus - Venkatesan	13-30
00467-02	Structure and Function of Polypeptides Encoded by AIDS RV Genome - Venkatesan	13-31

Public Health Service - National Institutes of Health
SUMMARY REPORT

ANNUAL REPORT OF THE LABORATORY OF MOLECULAR MICROBIOLOGY, NIAID
October 1, 1986 - September 30, 1987

Dr. Malcolm A. Martin, Chief

The Laboratory of Molecular Microbiology (LMM) applies molecular biological techniques to study the structure and regulation of prokaryotic and eukaryotic genes. Programmatically, a wide range (retroviruses, fungi, streptococci, anaerobic bacteria, and mycoplasmas) of microorganisms and their host cells are investigated. Further complicating matters, members of the LMM are located in two different buildings in Bethesda, and, during 1987, in three separate sites at the Frederick Cancer Research Facility while renovations to Building 550 are taking place. Although the research activities conducted by LMM staff are quite diverse, a common theme permeating most of the programs centers on the characterization of genetic elements (both chromosomal and episomal) involved in the interaction of microorganisms and the cells they infect.

For the past three years, the major research effort of the LMM scientific staff has focused on AIDS and the human immunodeficiency virus (HIV). Much of this work has been carried out as a collaborative effort with Drs. Tom Folks, Clifford Lane, and Anthony Fauci of the Laboratory of Immunoregulation, who provide expertise in cell biology and clinical immunology.

Analyses of the envelope genes present in different HIV isolates have indicated the presence of several highly variable and conserved domains. In the case of some African isolates, greater than 50% variability was noted at the deduced amino acid level involving stretches of more than 100 residues of the external envelope glycoprotein, gp120. Despite this degree of envelope heterogeneity, all HIV isolates tested have retained their tropism for $T4^+$ lymphocytes and their ability to induce cytopathic effects during productive infection in tissue culture. This suggests that one or more of the conserved domains within gp120 are involved in the adsorption of HIV to the CD4 receptor as well as in subsequent steps of viral replication. During the past year, site-specific mutagenesis techniques have been used to target potential N-linked glycosylation sites situated in the second conserved region of gp120. One of four HIV mutants examined was rendered noninfectious by this procedure. Interestingly, *in vitro* assays indicated that the mutant gp120 was capable of binding to the CD4 molecule and induced the formation of syncytia following incubation with $T4^+$ cells. This result implies that portions of the HIV envelope, not involved in the binding to CD4, may be necessary for the initiation of a productive viral infection. Interaction with other cellular surface proteins and/or processing of the bound virions may be required subsequent to their interaction with the CD4 receptor. Of note is a recent report from another laboratory demonstrating that the portion of gp120 principally involved in CD4 binding is located nearly 200 amino acid residues downstream from the glycosylation mutant.

An added bonus of this work was the discovery, in long-term cultures of the glycosylation mutant, of revertants which had retained the original mutation but had acquired compensatory changes at another site within gp120. A single amino acid substitution (serine to asparagine) was noted in several revertants that were cloned and sequenced. This "second site" revertant occurred in the absence of immunological pressure (i.e., in a tissure culture system) and very likely reflects copying errors associated with reverse transcription or DNA dependent RNA polymerization. Exploitation of this system may prove useful in studies of HIV envelope gene heterogeneity.

During the course of transfection studies with an infectious molecular clone of HIV (which demonstrated that virtually all cells could synthesize virus proteins and assemble infectious progeny particles following the introduction of viral DNA), experiments were initiated to ascertain whether some of the same human cell lines could be directly infected with virus. Productive infection of two colon carcinoma cell lines was initially demonstrated by in situ hybridization; an additional colon line was also found to be infectible by co-cultivating infected cells with T4$^+$ lymphocytes. The level of virus producing cells never exceeded 0.2%; one of the colon carcinoma cell lines continued to produce infectious particles during the entire 9 month observation period.

Despite numerous attempts, we were unable to unambiguously detect T4 proteins on the surface of susceptible colon cells using monoclonal antibodies in indirect immunofluorescence, flow cytometry, or immunoperoxidase assays. In contrast, the characteristic 3.0 kb T4-specific mRNA could be demonstrated in infectible cell lines, but not in those refractory to virus infection. The same 3.0 kb RNA was detected in a normal colon mucosal RNA preparation. It is therefore tempting to speculate that the unique susceptibility to HIV infection of human colon and rectal cells might represent a "portal of entry" for HIV. The establishment of a chronic infection at this site could ultimately result is dissemination of the virus to cells of the lymphocytic and monocytic series and, in some cases, lead to the development of the AIDS disease complex.

As discussed earlier, transfection experiments using a full-length infectious copy of the HIV provirus, revealed that progeny particles, capable in initiating a productive infection of T4$^+$ lymphocytes, could be produced in a variety of non-human cells including those of mice. No intracellular restriction to the expression of HIV apparently exists. Studies were therefore started to model one phase of HIV infection--the presence of integrated but unexpressed proviral DNAs capable of being activated by environmental or microbiological factors. Transgenic mice were therefore constructed containing the HIV long terminal repeat driving the chloramphenicol acetyl transferase (CAT) gene. Four independently derived transgenic mouse strains showed a consistent tissue pattern of high CAT expression in thymus, heart, tail, and lens epithelium; intermediate CAT levels in spleen; and no CAT activity in brain, kidney, liver, lymph nodes, monocytes and peripheral blood. When T-lymphocytes from transgenic animals were propagated in the presence of IL-2, a 6- to 18-fold increase in CAT expression was observed compared to resting cells. A similar augmentation of CAT expression was seen following the propagation of peripheral blood monocytes. Infection of dividing T-lymphocytes with human adenovirus 5 resulted in a 50-fold increase in CAT activity; cytomegalovirus infection of monocyte cultures led to a 15-fold increase in CAT expression. These results indicate that tissue, immunologic and virologic factors influence expression of the HIV long terminal repeat in these animals. This system represents a safe and potentially valuable method to evaluate the effects of cellular and viral transactivators on dormant HIV proviruses.

Since many individuals infected with HIV subsequently become coinfected with a variety of other microorganisms as their immune function deteriorates, the possibility exists that some of these opportunistic pathogens act as "cofactors" that stimulate the expression of AIDS virus, thereby altering the clinical course. Cotransfection experiments were therefore carried out utilizing the HIV long terminal repeat (LTR) linked to the chloramphenicol acetyl transferase (CAT) gene in the presence of a variety of distinct classes of DNA viruses. Molecular clones containing JCV, BKV, lymphotropic papovavirus (LPV), human andenovirus 5, bovine papillomarivus, type 1 herpes simplex virus (HSV-1), and varicella-zoster virus (VZV) sequences, increased expression directed by the HIV LTR. Primer extension assays, specific for HIV LTR mRNA, revealed increased levels following transfection with the HIV *tat* plasmid as well as with several of the DNA viruses. Virus-specific RNA expression paralleled the simulation of CAT activity. More than additive effects were observed at both the RNA and protein levels when *tat* plus HSV-1 or *tat* plus JCV DNAs were transfected into cells with the HIV LTR-CAT plasmid. Further analysis revealed that HSV immediate early gene segments ICP0 and ICP4 stimulated HIV LTR directed CAT expression whereas ICP27 had no effect. Evaluation of HIV LTR deletion mutations indicated that the target of the HSV-1 gene products was different from that responsive to the HIV *tat* protein.

The "A" (sor) gene of HIV is unique to lentiviruses and is expressed as 5.5 and 5.0 kb polyadenylated RNAs which encode a 23 kDa protein present in productively infected cells. The biological function of the HIV "A" gene product was examined by deleting a 620 bp segment from an infectious cloned proviral DNA. The mutation had no effect on viral gene expression or on the assembly or release of particles in transfection experiments; however, a 1000-fold reduction in infectivity of these particles was noted when they were used to establish a primary infection of $T4^+$ lymphocytes following cocultivation of the originally transfected cells. The "A" gene defect could be restored in transcomplementation experiments using an "A" gene cDNA clone. Cell-to-cell spread of HIV had previously been considered a possible explanation for the chronic nature of viral invection *in vivo* which persists despite the existence of an excellent antibody response. Our experiments with the "A" mutant clearly demonstrate that this is the principle mechanism of virus spread. Although not formally demonstrated, it must be assumed that wild-type HIV also has the capacity to spread cell-to-cell.

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RMOB
LPD
LVD

In a collaboration with Dr. Tom Folks (LIR), we had previously characterized a clonal cell line (8E5) derived from a population of lymphocytes that had survived acute infection with HIV. The 8E5 cells contained a single defective copy of HIV proviral DNA and produced non-infectious viral particles lacking reverse transcriptase (RT) and endonuclease proteins. The provirus was molecularly cloned from 8E5 cells and the functional defect was mapped to a 1931 bp region of the pol gene following a series of reciprocal exchanges between the mutant and an infectious molecular clone of HIV. The nucleotide sequence analysis of the mutant pol gene indicated that the defect was located within the segment encoding RT and was due to a 1 base deletion 301 codons from the amino terminus of RT. Expression of the 8E5 RT segment in *E. coli* generated an enzymatically inactive and truncated 33 kDa protein.

During the past three years, members of the LMM staff have established collaborative relationships with investigators at several medical schools and industry. One such arrangement with MicroGeneSys, a small biotechnology company located in West Haven, Connecticut, has involved the subcloning of HIV envelope genes in a baculovirus expression system for use as a potential vaccine. The advantages of the baculovirus system are twofold: 1) large quantities of protein can be generated in insect tissue cultures; 2) the proteins produced are glycosylated. This is therefore an ideal system for expressing the HIV env gene products, since gp160, gp120 and gp41 are all glycosylated. Our collaborators at MicroGeneSys have successfully expressed the envelopes of two HIV isolates in the baculovirus system. One of these (a gp160 preparation) has recently been injected into two chimpanzees and their sera is being tested for neutralizing activity in collaboration with Dr. Tom Folks (LIR). An IND application for this preparation of the HIV gp160 has been submitted to the FDA for vaccine trials in humans.

Other Research Accomplishments

Identification of HIV LTR sequences critical for transactivation by tat. Deletion mutations within the transactivation response region of the HIV LTR have identified sequences mapping between +24 and +38 as essential for the response of the HIV LTR to the viral transactivation protein, tat. In particular, a 4 bp deletion (+34 to +38) eliminates tat responsiveness. This region may represent a site of direct protein-nucleic aid interactions affected by tat. Alternatively, this 4 bp deletion dramatically alters the predicted RNA secondary structure of the 5' end of the mRNA and may thus alter mRNA stability. (Rabson).

Activation of integrated HIV proviruses. The ability of integrated HIV proviruses to be transcriptionally activated was demonstrated using a clone of the promonocytic cell line, U937. This clone, designated U1, contains two integrated HIV proviruses and constitutively expresses low levels of HIV. Treatment of U1 cells with cytokine-containing supernatants of PHA-stimulated lymphocytes and LPS-induced monocytes results in a 10- to 40-fold increase in HIV RNA. These cells may represent a model of activation of latent HIV infection by stimulation of the immune system. (Rabson, Clouse, Maury, and Folks)

Infection of placental-related tissue by HIV. Studies to define the mechanism of maternal-fetal transmission of HIV have resulted in the identification of mRNA and protein for the HIV receptor, CD4, in term human placenta. A human choriocarcinoma cell line, JEG, has been productively infected with HIV and may provide a model for transplacental spread of HIV infection. (Maury, Potts, and Rabson).

Identification of the HIV gag-pol protease Two forms (p64 and p51) of the HIV reverse transcriptase and a p34 integrase, all encoded by sequences located in the pol gene, have been previously identified and sequenced by our group. A p10 protease has now been identified and partially sequenced. The N-terminus of this 99 residue protein is situated 69 codons upstream of the pol open reading frame (ORF), utilizing the C-terminal 12 amino acids of the gag ORF. In metabolic labeling experiments, a novel 32 kDa protein was identified which contained tryptic peptides mapping to the protease domain of HIV. These peptides are being further characterized to localize the position of the gag-pol frameshift. (Venkatesan, Lillehoj, Mervis, and Ahmad).

Functional analysis of the HIV "B" and art proteins. Expressible cDNA plasmids were constructed for the functional analysis of the 27 kDa "B" and 14.5 kDa art gene products. The proteins encoded by the two cDNAs were verified by cell-free translation and partial amino acid sequencing. Both cDNA expression plasmids were utilized in co-transfection experiments involving mutant proviruses containing point mutations in the art or "B" ORFs. (Venkatesan, Ahmad, Mervis, and Lillehoj).

Analysis of leukemogenic determinants of MCF MuLV. A recombinant provirus was constructed by substituting a SalI-EcoRI segment, containing the integrase gene of the leukemogenic MCF 13 MuLV, with the analogous segment from the non-leukemogenic MCF 111A MuLV DNA. The recombinant virus obtained from transfection studies was injected into newborn AKR mice to determine its leukemogenic potential. Tumor development was delayed and the incidence of thymomas was reduced by 50%. These results suggest that the integrase gene of MCF viruses contributes to their leukemogenic potential. (Khan, Hartley, Austin, and Theodore).

Expression of a novel full-length endogenous MCF-related RNA species in autoimmune mouse strains. Endogenous ecotropic, xenotropic-like, and MCF env-related MuLV sequences are involved in the generation of recombinant leukemogenic MCF viruses. Previous studies have shown that spliced, but not full-length, MCF env-related mRNAs were expressed in mouse tissues suggesting that endogenous MCF-related proviruses might be replication-defective. Northern blot analysis of thymic tissues from four autoimmune mouse strains indicated that full-length (8.4 kb) transcripts of endogenous MCF env-related proviruses were present. This RNA species was absent in non-autoimmune mouse strains. The regulation of expression of endogenous MuLV sequences in autoimmune versus non-autoimmune mouse

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RMOB
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LVD

strains and the role of the novel viral transcripts in autoimmune disease are currently being investigated. (Khan, Kreig, and Steinberg).

Genetic mapping of chromosomal genes involved in viral oncogenesis. Hamster and mouse somatic cell hybrids and genetic crosses were analyzed to chromosomally localize proviral genes, cellular oncogenes and tumor-specific integration sites. Mouse mammary tumor (MMTV) proviruses were mapped to chromosomes 4 and 8 and the nomenclature system used for these genes was revised and standardized. The mouse homolog of the bcl-2 ocogene was mapped to chromosome 1, and tumor specific integration sites were mapped to chromosomes 4, 17, and 19. This program in genetic mapping has also been greatly expanded resulting in the mapping of many genes such as those encoding Il-3, the immume interferon receptor and various enzymes. (Kozak).

Characterization of a novel leukemogenic MuLV from Mus hortulanus. A novel infectious ecotropic MuLV (HoMuLV) has been isolated from the wild mouse species M. hortulanus. HoMuLV differs from other naturally occurring ecotopic MuLVs in several biological properties, in its restriction map, and in its ability to induce lymphomas in female mice. Southern blot hybridization and preliminary nucleic acid sequencing indicate that HoMuLV contains a novel envelope gene. (Kozak, Voytek).

Expression of nonecotropic MuLV sequences in wild mice. Virus expression was examined in wild mice which contain endogenous copies of MCF or xenotropic env-related genes. Most mice synthesize a characteristic 3.0 kb env mRNA, but some European mice produce an unusually small 2.0 kb MCF-related message. M. m. domesticus produces a full-length 8.4 kb RNA, but no infectious virus could be isolated; however, infectious MCF virus was isolated five weeks after inoculation with ecotropic MuLV, indicating that the endogenous sequences could contribute to the generation of replication competent MCF virus. M. spretus failed to produce any detectable mRNA despite the presence of ten integrated MCF-related proviral DNA sequences. Experiments are underway to determine if lack of expression is due to the presence of a resistance gene such as Gv-1. These mice produced MCF-related 3.0 and 8.4 kb mRNAs, as well as infectious virus, following inoculation with ecotropic MuLVs, suggesting that recombinant viruses can be generated in the absence of detectable transcription from endogenous sequences. (Kozak, Villar)

Site directed mutagenesis of conserved amino acid residues in MoMuLV reverse transcriptase. Five amino acids conserved in all retroviral reverse transcriptases have been altered by site specific mutagenesis in a MoMuLV model system. Cloned MoMuLV DNA, containing any one of the altered amino acid codons, is unable to produce infectious particles in transfected NIH3T3 cells. Infectious MoMuLV, however, can be recovered by co-transfection with MCF or amphotropic MuLV proviral DNA. One alteration, an aspartic to glycine mutation in MoMuLV, results in a greater than 10-fold reduction of RNase H activity without concomitant effect on reverse transcriptase. Preliminary results with two other mutations suggest both RNase H and reverse transcriptase activities are significantly reduced. These results indicate the identified conserved sequence participates in RNase H and reverse transcriptase functions. (Repaske).

Homo-oligomeric structure of p21 ras proteins. Using radiation inactivation techniques, it was shown that the products of ras genes exhibit an oligomeric target size when assayed both functionally and structurally. Such a structure has important implications for models of p21 as a signal transducer. These results suggest that activation of p21 occurs through allosteric subunit reorientation within the oligomer upon guanine nucleotide binding. Cross-linking experiments allowed identification of a cross-linked species corresponding closely to the target size predicted by radiation inactivation. (Alonso, Nebreda, Santos).

Role of ras gene products in the phosphoinositide pathway. The components of this pathway were similar in normal cells and in cells transformed by ras or other "cytoplasmic" oncogenes. "Nuclear" oncogenes did not induce demonstrable changes in this pathway. These results exclude a role of p21 as a G protein for phospholipase C, the key regulatory enzyme in the signal pathway. Two biochemical markers for malignant transformation were identified: a) a specific stimulation of phospholipase A2 measured by the index GPI/IP and b) a biochemical uncoupling to external stimuli as indicated by the absence of Ca++ release from the ER in the presence of bombesin or PDGF. (Alonso, Santos).

Studies on the dissemination of Streptocococcal antibiotic resistance determinants. Three distinct genes encoding resistance to kanamycin (Km), streptomycin (Sm), and erythromycin (Em), originally cloned from plasmid pJH1, are normally plasmid-mediated in multiply resistant streptococci isolated from human and animal sources. One Km, Sm, and Em resistant strain of Streptococcus faecalis, LDR175, isolated from chicken, transferred this multiple resistance phenotype to a plasmid-free S. faecalis strain by filter mating. One of the transconjugants, strain LDR517, as well as strain LDR175, contained all three genes in their respective chromosomes. A strain of S. faecalis harboring pJH1, DL77, and strain LDR517, mediated the translocation of the three resistance genes to pAD1, a hemolysin-bacteriocin plasmid, following the latter to each strain, on a common DNA segment greater than 13 kilobase pairs in size. This putative multiple antibiotic resistance transposon is being characterized. A new tetracycline resistance (TcR) gene, tetO cloned from a pig isolate of Streptococcus mutains, exhibited no DNA sequence homology to the three previously described streptococcal TcR genes, tetl, tetm, and tetn. Teto did hybridize, under stringent conditions, to DNA from several tetracycline resistant Campylobacter strains isolated from humans and chickens, and to a TcR determinant cloned from a plasmid isolated from a strain of Campylobacter jejuni. (LeBlanc and Lee).

Functional and structural analysis of streptococcal broad host-range plasmids. A 5.0 kb EcoRI fragment from the 26 kb erythromycin resistance plasmid, pAMB1, was shown previously to replicate in Streptococcus sanguis. This 5.0 kb fragment was cloned in both orientations in *E. coli* using pUC19 or M13mp19. Deletions were produced from each end of the 5.0 kb replicon, at approximately 50 base pair intervals, by digestion with exonuclease III. The deleted fragments in M13 are being used as substrates for sequence determinations by the dideoxy chain termination procedure. The pUC19 deletion derivatives are being cleaved from the vector, blunt-end ligated to differentially selectable streptococcal antibiotic resistance genes, and used to transform *S. sanguis*. The latter molecules will be used to locate copy number and incompatibility regions on the replicon, and to define the minimal size required for replication. The same approach was also used to initiate studies on the 4.2 kb cryptic streptococcal plasmid, pVA380-1, which has been used as a cloning vector in several streptococcal host-vector systems. (LeBlanc, Lee and Zito).

Transposon Mutagenesis. Tn916 is a 15 kb tetracycline resistance (TcR) transposon containing genes for conjugation in the absence of plasmid DNA. The transposon was transferred from *Streptococcus faecalis* to *Bacillus anthracis* and to virulent strains of *Streptococcus agalactiae* (group B Streptococcus) by a filter mating procedure. The TcR trait was transferred to both recipient species at frequencies of 10^7 to 10^8. Southern blot hybridizations using *Hind*III digested DNA from the transconjugants and ^{32}P-labeled Tn916 DNA as a probe indicated multiple and random insertions of the transposon into chromosomal DNA of *B. anthracis* and *S. agalactiae*. Among the *B. anthracis* Tn916-containing transconjugants, mutations to amino acid auxotrophy appeared at frequencies of greater than 1%. The *S. agalactiae* transconjugants are being tested for the loss of adherence to fetal epithelial cells, a trait associated with virulence of group B Streptococcus. (LeBlanc).

Regulation of ermFS expression in Tn4551. Studies of gene regulation and dissemination in the anaerobic pathogen *Bacteroides fragilis* continued to focus on the clinically significant clindamycin resistance determinant (*erm*FS) and its associated IS element, IS4351 that activated transcription of *erm*FS. These results coupled with *in vitro* mutagenesis served to identify the major *erm*FS promoter. Using Northern blot analysis, a second *erm*FS promoter was located upstream of the major promoter. This second promoter activated transcription of a bicistronic message composed of the putative IS4351 transposase plus *erm*FS. (Smith).

Structural analysis of Tn4551. Tn4551 is a composite transposon structure bounded by 1.2 kb direct repeat sequences. DNA sequence analysis revealed that the repeat was structurally similar to a number of known IS elements and was designated IS4351 due to its relationship to the repeats found on Tn4351. The element was 1155 bp in length and contained a large ORF of 978 bp. Comparisons with other IS elements revealed weak amino acid homology between the large ORF and the transposase of IS3. Preliminary examination of the transcription units of IS4351 revealed the presence of two major mRNAs one of which encoded the putative transposase. Further results suggested that the levels of this transposase mRNA may be regulated by the presence of additional copies of IS4351. Studies designed to demonstrate a relationship between the regulation of this RNA and transposition are presently underway. (Smith)

Molecular probes for detection of Mycoplasma genitalium. Because of difficulties in cultivating *Mycoplasma genitalium* from human clinical specimens, two types of molecular probes were developed to detect organisms in experimentally-infected chimpanzees. An immuno-binding assay with polyclonal or monoclonal antisera directed to the 140 kDa attachment protein could detect about 10,000 organisms. A radioactive DNA probe, specific for *M. genitalium*, could detect 0.1 nanogram of DNA (equivalent to approximately 1000 organisms) in dot blot hybridizations. (Tully, LeBlanc, Baseman, Razin).

Biological and molecular features of mollicutes. A large group of wall-less prokaryotes (mollicutes or mycoplasmas), primarily of insect origin, have been characterized in a variety of biological and genetic tests to evaluate their potential in genetic transformation procedures. A plasmid (pRA1) recovered from a spiroplasma has been shown to carry short, interspersed, and repetitive DNA sequences that are also found in chromosomal DNA of three important plant pathogenic spiroplasmas. It was also demonstrated these same DNA fragments hybridized to DNA obtained from some type 3 spiroplasma viruses found in these organisms. The significance of these shared DNA sequences among spiroplasma chromosomes, plasmids, and viruses is presently unknown. (Tully, LeBlanc, Nur, Ranhand)

Honors and Awards

Malcolm Martin

Recipient: HHS Distinguished Service Award, May 4, 1987.

Invited speaker: Lineberger Cancer Research Center, University of North Carolina, October 8, 1986.

Invited participant: NAS/IOM Workshop on Human Somatic Cell Gene Therapy, Washington, D.C., October 16, 1986.

Member, WHO Study Group on Biologicals, Geneva, Switzerland, November 18-19, 1986 Meeting.

Invited speaker: Pennsylvania State University School of Medicine, Hershey, Pennsylvania, February 12, 1987.

Invited speaker: Workshop on Virus Infection of Immunocytes, Immunological Dysregulation, and CNS Disease, Coronado, California, March 4-7, 1987.

Invited speaker: UCLA Symposium on Human Retroviruses, Cancer and AIDS, Keystone, Colorado, April 1-6, 1987.

Keynote speaker: Mosbach Colloquium, Mosbach, Germany, April 9-11, 1987.

LMSF
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LPVD
RMOB
LPD
LVD

Invited speaker: 2nd ZMBH Forum on Molecular Biology, Heidelberg, Germany, May 22-23, 1987.

Member, Organizing Committee, Wallace Rowe Symposium on Animal Viruses, February, 1987.

Member, Program Committee and Session Chairman, III International AIDS Conference, Washington, D.C., June, 1987.

Member, PHS AIDS Vaccine Research and Development Subgroup.

Member, Scientific Review Board, Lucille P. Markey Charitable Trust, Miami, Florida.

Member, Editorial Board, Journal of Virology.

Joseph Tully

Associate Editor, International Journal of Systematic Bacteriology.

Member, Culture Collection Committee, American Society of Microbiology, (1985-)

Archivist, International Organization of Mycoplasmology. Co-Director, International Mycoplasma Techniques Course on Molecular Biology, Bordeaux, France, June 22-July 8, 1987.

Invited speaker: Seminar on "Mycoplasmas and Human Genital Tract Infections," American Society of Microbiology, Atlanta, Georgia, March 3, 1987.

Donald LeBlanc

Invited Lecturer: University of Texas Health Science Center at San Antonio, Department of Microbiology, San Antonio, Texas, January 8, 1987.

Invited Lecturer: Department of Biology, Mercy College, Dobbs Ferry, New York, April 8-9, 1987.

Christine Kozak

Invited speaker: Wallace Rowe Symposium on Animal Viruses, Bethesda, Maryland, February 2, 1987.

Organizer and Chair: Committee to Standardize the Nomenclature for Endogenous MMTVs.

Jeffrey Smith

Invited speaker: East Carolina Medical School, Department of Microbiology and Immunology, Greenville, North Carolina, June 1987.

Animal Molecular Biology Study Panel; USDA competitive grants program, May 1987.

Eugenio Santos

Invited speaker: Spanish Research Council, Madrid, Spain, April 1987

Keynote speaker: Spanish XVII Microbiology Congress, Gijon, Spain, June 1987.

Arnold Rabson

Associate Editor: Human Retroviruses and AIDS, 1987.

Invited speaker: Nordic Research Symposium on Human Retroviruses; Mariehamn, Finland, March 1987.

Arifa Khan

Invited speaker: Workshop on Pathogenesis by Non-acute Retroviruses, Irvine, California, October, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00027-20 LMM

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Basic Studies of Mycoplasmas

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Joseph G. Tully	Head, Mycoplasma Section,	LMM, NIAID
Others:	David L. Rose	Research Microbiologist	LMM, NIAID

COOPERATING UNITS (if any) J.B. Baselman, Univ. Texas, San Antonio, TX; J.M. Bove, Univ. Bordeau, France; S. Razin, Hebrew Univ. Jerusalem; R.F. Whitcomb, USDA, Beltsville, MD; I. Nur, NIADDKD; D.J. LeBlanc and J. Ranhand, LMM.

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Mycoplasma Section

INSTITUTE AND LOCATION
NIAID, NIH, Frederick, Maryland 21701

TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 1.0	OTHER: 2.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recent efforts involve further characterization of the biological and molecular features of Mycoplasma genitalium, in anticipation that this information will lead to development of improved methods in detection of the organism and more definite confirmation of its role in human urogenital disease. The identification of a unique attachment (surface) protein (molecular wt. 140 kDa) on the organism differentiates it clearly from other pathogenic mycoplasmas with organized terminal structures and identifiable attachment proteins. Cross-reactive as well as species-specific proteins of M. genitalium and M. pneumoniae have been identified using monoclonal antibodies generated against these mycoplasmas in ELISA and Western blot analyses. An immuno-binding test, using mycoplasma blotted onto nictrocellulose paper and monoclonal or polyclonal antibody preparations to M. genitalium as an immunologic probe for detection of the organism, proved specific but with inadequate sensitivity for use as a practical diagnostic test . In collaboration with investigators in Israel and LMM, a radiolabeled DNA probe specific for M. genitalium has been used in dot blot hybridizations. The probe can detect 0.1 nanogram of M. genitalium DNA and approximately 1000 organisms, and does not hybridize with other mycoplasmas found in the human urogenital tract. In other work, biological and molecular features of a large group of mollicutes (wall-less prokaryotes) were examined for their potential in genetic transformation studies. A plasmid (pRA1) obtained from a spiroplasma in this group has been shown to possess short, interspersed, and repetitive DNA sequences that are also found to hybridize to chromosomal DNA form three plant pathogenic spiroplasmas. It was also demonstrated that these same DNA fragments exhibited homology to DNA obtained from certain type 3 spiroplasma viruses. The biologic function of these common sequences among spiroplasma chromosomes, plasmid and viruses is not presently understood.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00190-09

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Molecular Genetics of Eukaryotic Cells and Their Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Malcolm A. Martin	Chief	LMM, NIAID
Others:	Akio Adachi	Visiting Fellow	LMM, NIAID
	Howard Gendelman	Expert	LMM, NIAID
	John Leonard	Medical Staff Fellow	LMM, NIAID

COOPERATING UNITS *(if any)*

Thomas Folks, Expert, LIR, NIAID; Heinrich Westphal, Section Chief, LMG, NICHD; Jaspal Khillan, Visiting Fellow, LMG, NICHD; Monte Meltzer, Immunologist, WRAIR

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Thirteen adherent human non-lymphocyte cell lines were tested for their susceptibility to infection by the human immunodeficiency virus (HIV). Productive infection could be demonstrated in three of five colorectal carcinoma cell lines examined; the other eight human lines were not infectible. A susceptible colon carcinoma cell line (HT29) as well as normal colonic mucosa was shown to contain a 3.0 kb species of poly(A)+ CD4 RNA, whereas uninfectible colon carcinoma and rhabdomyosarcoma cell lines synthesized no detectable T4 RNA. A persistently infected colon carcinoma cell line was established that continued to produce progeny HIV for nine months.

In collaboration with Dr. Heinrich Westphal, NICHD, transgenic mice were constructed that contain the HIV long terminal repeat (LTR) linked to the gene for the bacterial enzyme chloramphenicol acetyltransferase (CAT). Independently derived transgenic animals showed a consistent tissue pattern of high CAT expression in thymus, heart, tail and lens epithelium. When T-lymphocytes from transgenic animals were propagated in the presence of IL-2, a 10-fold increase of CAT activity was observed. This system represents a safe and potentially valuable model for evaluating the effects of cellular and viral transactivators on dormant HIV proviruses.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00218-06 LMM

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biochemical and Chemical Studies on Retroviral DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Roy Repaske	Research Chemist	LMM, NIAID
Others:	Christine Kozak	Microbiologist	LMM, NIAID
	Malcolm Martin	Chief	LMM, NIAID

COOPERATING UNITS *(if any)*

Janet Hartley, Research Microbiologist, LVD, NIAID

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Biochemical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Alignment of pol gene deduced amino acid sequences of retroviruses of man (HIV-1, II), ape, cow, sheep, mouse, and chicken were made to identify conserved regions, presumably functional domains, of the enzyme reverse transcriptase. Although little overall sequence conservation was found, one short, highly conserved domain was identified and selected for further study. Site-directed mutagensis was employed to alter these conserved amino acid codons and the effects of these changes were measured biologically and biochemically. Moloney murine leukemia virus (MuLV) was used as the model system. Alteration of four of the five individual amino acids resulted in inability of the transfected modified cloned DNA to produce infections virus without use of helper virus. The biochemical effect of these mutations on reverse transcriptase (RT) and the associate RNaseH activity was determined on purified mutant RT expressed in E. coli. One mutation caused a greater than 10 fold decrease in RNase H activity without simultaneously altering RT activity. Other mutant RTs had depressed RT and RNaseH activities.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00219-06 LMM

PERIOD COVERED
October 1, 1986 to September 30, 1987.

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular and Genetic Analysis of Streptococci

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.J. LeBlanc	Head, BVS,	LMM, NIAID
Others:	L.N. Lee	Chemist,	LMM, NIAID
	E. Zito	NRC Postdoctoral Fellow	

COOPERATING UNITS *(if any)*
Dr. Bruce Ivins, Division of Bacteriology, USAMRIID, Fort Detrick, Frederick, MD; Dr. Stephen Mattingly, Department of Microbiology, University of Texas Health Science Center at San Antonio, TX.

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Bacterial Virulence Section

INSTITUTE AND LOCATION
NIAID, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	0.5	1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The properties and molecular structures of two broad host-range streptococcal plasmids are being studied in detail. A 5.0 kbp EcoRI fragment from pAMβ1, shown previously to contain all that is necessary for replication in Streptococcus sanguis, has been cloned in both orientations on pUC19 and on phage M13mp19. A series of nested deletions, in both directions, were obtained by Exonuclease III digestion. The deletions of the M13 clones are currently being used for the nucleotide base sequence analysis of the 5 kbp replicon. The deletions of the pUC19 clones are being cleaved from this vector, blunt-end ligated to streptococcal DNA fragments containing selectable antibiotic resistance determinants, and used in transformation experiments with the Challis strain of S. sanguis. These resistance gene-tagged deletion derivatives are being used to locate regions of this replicon associated with copy number control and incompatibility traits. Similar experiments were also conducted with the 4.2 kbp cryptic plasmid, pVA380-1, which has been used extensively as a cloning vector in streptococcal host-vector systems. Collaborative studies on the use of transposon mutagenesis for genetic and molecular analyses of virulence traits in the Gram-positive pathogens, Bacillus anthracis (with Dr. B. Ivins) and Streptococcus agalactiae (with Dr. S. Mattingly), were initiated. Tn916, a conjugative tetracycline resistance transposon from Streptococcus faecalis, was successfully transferred to strains of both species and was also shown to insert at several locations on their respective chromosomes. Auxotrophic mutants of B. anthracis Tn916-containing transconjugants were obtained.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00222-06 LMM

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characterization of Endogenous Ecotropic and Xenotropic Murine Leukemia Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Theodore Theodore	Research Microbiologist	LMM, NIAID
Others:	Arifa S. Khan	Senior Staff Fellow	LMM, NIAID
	Malcolm A. Martin	Chief	LMM, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Biochemical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mink cells focus forming (MCF) viruses are dualtropic murine leukemia viruses (MuLVs) which have been isolated form leukemic mouse tissue. These viruses have been divided into two classes based on their ability to accelerate the onset of thymic lymphomas in AKR mice. Both classes of MCF viruses arise by recombination involving ecotropic MuLV and endogenous MCF-related env sequences. Leukemogenic MCF MuLVs (class 1) replicate efficiently in the thymus whereas the non-leukemogenic (class II) grow poorly in the thymus. Long terminal repeats (LTRs) have been shown to be involved in tissue-specificity and disease induction associated with leukemogenic MuLVs; also, sequences present in the gag, pol, or env regions, have been shown to be necessary for expression of maximum leukemogenicity. To study the sequences of MCF-MuLVs involved in viral leukemogenesis, molecularly cloned DNAs of leukemogenic MCF-13 and non-leukemogenic MCF-111A MuLVs were obtained and the nucleotide sequences of the LTRs, 3' pol and 5' env regions determined. The results of comparative analysis indicated differences in the LTR and 3' pol regions between MCF-13 and MCF-111A MuLV DNAs. The LTR associated with MCF-13 was closely related to that present in xenotropic MuLVs, whereas the LTR sequence of MCF-111A was identical except for 1 bp to the ecotropic proviral LTR. A 12 bp nucleotide stretch, characteristic of leukemogenic MCF MuLVs, was present in MCF-13 in the 3' pol region but was lacking in MCF-11A. No significant sequence divergence was seen between MCF-13 and MCF-111A MuLV DNAs in the env region. These results suggest that the leukemogenic potential of MCF-13 may reside in LTR and 3' pol sequences.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00281-06 LMM

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Cloning of Recombinant MuLV Proviral Sequences in Inbred Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Charles E. Buckler	Research Biologist	LMM,NIAID
Others:	J. Silver	Medical Officer	LMM,NIAID

COOPERATING UNITS *(if any)*
None

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SECTION
Viral Biology Section

INSTITUTE AND LOCATION
NIAID. NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0	0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The study the molecular nature of the mouse genetic locus, Fis-1, has been continued. This locus has been shown to be the site of integration of Friend MuLV in some lymphomas appearing after inoculation of several inbred mouse strains with the ecotropic Friend MuLV. In about 5% of approximately 100 tumors examined, copies of Friend MuLV were found integrated within a 1.5kb region of the mouse genome (Fis-1). From a mouse gemonic library constructed in lambda virus, 35kb of mouse genomic sequence surrounding the Fis-1 locus were isolated. These mouse sequences were present in two recombinant lambda phages. The mouse sequence inserts in the lambda phage were subcloned into plasmids and fragments from 7 to .3 kb were obtained. Each subcloned fragment was anayzed for unique or repetitive mouse sequence content by Southern blot analysis. Subclones containing a total of about 15kb of unique mouse sequence were further utilized to test for expression in RNA isolated from mouse tumors. Gene mapping experiments show that Fis-1 is extremely closely linked to Int-2, a preferred integration region for mouse mammary tumor virus in mammary carcinomas. Studies at the RNA and DNA level prove that these loci are distinct.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

701 AI 00300-06 LMM

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Control of Resistance to Viral Leukemogenesis in Wild Mouse Populations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	C. Kozak		LMM, NIAID
Others:	R. O'Neill	Staff Fellow	NINCDS, NIH
	P. Voytek	Guest Worker	LMM, NIAID
	C. Villar	Guest Worker	LMM, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	3	1

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The genetic basis of disease induction by murine leukemia viruses (MuLVs) has been studied largely by analysis of the different inbred strains of the laboratory mouse. However, wild mice show a greater genetic diversity than the inbred strains, and our use of these animals has expanded our understanding of viral leukemogenesis in several important ways. First, direct analysis of wild mice shows that they differ markedly from the inbred strains in the number and type of endogenous MuLVs they carry. We have now shown that xenotropic and MCF-related MuLV env genes are present as endogenous copies in different populations of wild mice. We have also shown that amphotropic MuLVs are transmitted as exogenous virus in Southern California mice and are not present as germline copies in any mammalian DNA tested. Second, infectious viruses from wild mice differ from their laboratory mouse counterparts at the molecular level and have different patterns of pathogenesis. Transcription of endogenous MuLV sequences has been examined in various wild mice, and a new MuLV isolate, HoMuLV, has been shown to be leukemogenic in inbred strains.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00301-06 LMM

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cellular Genes Involved In Transformation and MuLV Transmission in Inbred Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Kozak	LMM, NIAID
Others:	P. Callahan	LTIB, NCI
	J. Ihle	NCI-FCRF, BRI-Basic Res.
	R. Lazzarini	LMG, NINCDS
	S. O'Brien	LVC, NCI
	D. Nebert	LDP, NCI

COOPERATING UNITS *(if any)* P. Curtis, Wistar Inst., Phila.; P. Jolicoeur, Clin. Res. Inst., of Montreal Canada; S. Pestke, Roche Inst. Nutley, NJ; D. Steffen, Worcester Fdn. for Exper. Biol., Shrewsburg, MA; C. Croce, Wistar Inst., Phila. PA; N. Shaper, Cooperative Units listing continues on page 3.

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Genetic studies on retrovirus-induced disease and the transmission of infectious virus have led to the identification of numerous chromosomal genes involved in these processes. Genetic mapping of these genes to specific chromosomal loci has been accomplished by the use of somatic cell hybrids alone or in conjunction with classical Mendelian crosses. These studies have now led to the chromosomal mapping of common provirus integration sites found in tumors induced by Gross Passage A MuLV, (Gin-1) Moloney MuLV (Dsi-1) and mouse mammary tumor virus (Int-3). Because these studies have also mapped an increasing number of germline viruses, a revised nomenclature system was devised for one group of endogenous retroviruses, the MMTVs. Use of hybrid cells also permitted the identification of the Bxv-1 provirus and implicated this provirus in the formation of the leukemogenic MCF MuLV. Finally, cell hybrids have been increasingly used to chromosomally map other genes for which cloned probes are available including an interferon receptor, interleukin-3 and the oncogenes Draf, Ets, Rel, and Mbcl-2.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00304-06 LMM

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Pathogenesis of Disease Induced by Friend MuLV

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Jonathan Silver Medical Officer LMM, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

F-MuLV can induce several different hematopoietic neoplasms including T-and B-cell lymphoma, erythroleukemia and myeloid leukemia. As one approach to studying disease pathogenesis in this system, we cloned sites of F-MuLV provirus insertion in host DNA in an F-MuLV-induced myeloid leukemia. One 2.5 kb region indentified in this manner was found to contain an F-MuLV provirus in one other myeloid leukemia and three thymic lymphomas out of a total of 90 tumors exmained. This result indicated that proviral insertion sites in F-MuLV-induced tumors in host DNA are not random. It is likely that provirus insertion in this common integration reigon, designated Fis-1, confers a growth advantage. We mapped the FIS-1 locus to mouse chromosome 7, about 26 cM distal to the locus for beta globin. Surprisingly, we found that Fis-1 mapped to the same position as Int-2, a putative oncogene involved in murine mammary carcinomas. Nevertheless, Fis-1 and Int-2 are distinct since 30 kb of DNA surrounding Fis-1 do not overlap 30 kb surrounding Int-2, and proviral insertions in Fis-1 do not induce Int-2 mRNA. This Fis-1/Int-2 region may be involved in human B cell neoplasia since the human homologue of Int-2 has been mapped to the same chromosomal band as a locus involved in B cell lymphoma, Bcl-1. We are currently using the technique of pulse-field and reverse-field electrophoresis to generate large scale restriction maps of the Fis-1/Int-2 region of mouse chromosome 7 determine how close these loci are to one another on a molecular scale and to search for an oncogene induced by proviral insertions at Fis-1.

PHS 6040 (Rev 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00353-05 LMM

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structural and Functional Analysis of Endogenous Proviruses of Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.S. Khan	Senior Staff Fellow	LMM, NIAID
Others:	J. Hartley	Chief, Viral Oncology Section	LVD, NIAID
	J. Austin	Microbiologist	LVD, NIAID
	T. Theodore	Research Microbiologist	LMM, NIAID
	A. Steinberg	Chief, Cellular Immunology Sec.	NIAMD
	A. Krieg	Medical Staff Fellow	NIAMD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Biochemical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	1.7	.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mouse genome contains at least 50 copies of murine leukemia viral (MuLV)-related DNAs, the majority of which are defective. Recombination between different infectious and defective MuLV sequences results in the generation of novel MuLVs including lymphomagenic mink cell focus-forming (MCF) viruses. The main goals of this project are: 1)to study the structure and regulation of expression of endogenous defective MuLV proviral DNAs; 2)to study generation of recombinant MCF viruses; and 3)to identify sequences contributing to leukemogenicity of MCF MuLVs.

Full-length (8.4 kb) transcripts of endogenous MCF env-related proviruses were highly-expressed in thymus tissues of autoimmune mouse strains but were not detectable in nonautoimmune strains. The 8.4 kb RNA species was detected from day 1 of life in NZB mice, prior to clinical manifestations of autoimmune disease. The data indicates that the expression of endogenous MCF-related DNAs is regulated differently in autoimmune versus non-autoimmune mouse strains.

An MCF LTR-specific DNA probe was constructed to identify MuLV proviruses which could donate LTR sequences to generate recombinant MCF MuLVs. Two potential LTR-progenitors were detected: one contained xenotropic env-related sequences and was chromosomally mapped as the inducible, endogenous xenotropic MuLV locus, Bxv-1; the second was characterized as a novel endogenous MuLV provirus which was related in env to MCF MuLVs. The latter MuLV DNA was conserved in the genomes all inbred and several wild mice.

To study the contribution of the integrase gene in MCF-induced thymomas, a 2.7 kb DNA segment containing the 3' pol region of leukemogenic MCF13 was substituted by an analogous segment from non-leukemogenic MCF111A MuLV DNA. The recombinant virus obtained from transfection studies was injected into newborn AKR mice to test whether the MCF viral genome retained its leukemogenic potential with the substituted integrase gene. Tumor development was delayed and the incidence of thymomas was reduced by 50%. These results suggest that the integrase gene contributes to leukemogenicity of MCF viruses.

LMSF
LPB
LPVD
RMOB
LPD
LVD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00388-04 LMM

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Functional Analysis of Human Retroviral Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Arnold Rabson	Senior Staff Fellow	LMM, NIAID
Others:	Wendy Maury	IRTA	LMM, NIAID
	Barbara Potts	Senior Staff Fellow	LMM, NIAID
	Malcolm Martin	Chief	LMM, NIAID
	Thomas Folks	Expert	LIR, NIAID
	Kathleen Clouse	Assistant Professor	Dept. of Microbiology Georgetown U.

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Biochemical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1.5	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The primary focus of this project over the last year has been the study of the regulation of the expression of the Human Immunodeficiency Virus (HIV). The goal of the project is the elucidation of the viral and cellular factors that may regulate the activation of HIV expression thus contributing to the pathogenesis of AIDS.

Our studies of the regulation of HIV expression have focused on the following systems: (1) we have studied DNA sequences within the HIV LTR that respond to the viral transactivation gene, tat and have identified a four-base pair segment crucial for tat activity; and (2) we have analyzed the activation of HIV expression in promocytic cells constitutively producing low levels of HIV and have shown that lymphokines and monokines can induce high levels of HIV expression in these cells.

An additional series of studies has addressed the problem of transplacental infection by HIV. We have demonstrated that the human placenta contains CD4 mRNA and protein. Furthermore, we have productively infected a human choriocarcinoma cell line with HIV. These studies suggest that prenatal transmission of HIV may occur through infection of placental tissue.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00395-04 LMM

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic and Molecular Analysis of Anaerobic Bacteria Indigenous to Humans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: C. Jeffrey Smith Sr. Staff Fellow LMM, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Bacterial Virulence Section

INSTITUTE AND LOCATION
NIAID, NIH, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Antibiotic resistance plasmids encoding resistance to the clinically significant drug clindamycin (Cln), were used as model systems for the analysis of gene expression and the dissemination of genetic information in the pathogen *Bacteroides fragilis*.

The Cln resistance determinant, ermFS, located within Tn4551 (on plasmid pBI136) was studied by gene cloning and DNA sequence analysis. The results were used for the generation of ermFS operon fusion vectors to study promoter activity and structure in IS4351. IS4351 bounds the composite transposons Tn4351 and Tn4551; studies indicate that transcription of ermFS is controlled by the IS elements. Data obtained with the operon fusion vectors located a significant promoter within the IS element that directed transcription toward ermFS in Tn4551. This location was verified by *in vitro* mutagenesis of the putative promoter followed by concomitant loss of ermFS activity in the fusion vector. Northern Blot analyses of ermFS tran-scripts confirmed these data but also revealed the presence of a second promoter within IS4351.

The complete nucleotide sequence of IS4351 was determined and the results indicated that the element was 1155 bp in length with two 25 bp regions of imperfect dyad symmetry at its termini. The element was dominated by a large open reading frame which possessed weak amino acid homology to the transposase of IS3. Initial studies on the role of IS4351 in Tn4551 transposition and its regulation have involved the use of Northern Blot analysis. These results showed that plasmids bearing single copies of IS4351 had high levels of the putative transposase message. In the presence of two IS copies, transcription of the transposase was not detected suggesting the possibility that the regulation of Tn4551 transposition is at the level of transcription.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00415-04

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
The Molecular Biology of Retroviruses Associated with AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Pnncipal Investigator.) (Nema, title, laboratory, and institute affiliation)*

PI:	Malcolm A. Martin	Chief	LMM, NIAID
Others:	Klaus Strebel	Guest Researcher	LMM, NIAID
	Barbara Potts	Senior Staff Fellow	LMM, NIAID
	Thomas Klimkait	Guest Researcher	LMM, NIAID

COOPERATING UNITS *(if any)*

Thomas Folks, Expert, LIR, NIAID; Jeffrey Ostrove, LCI, NIAID

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Biochemical Virology

INSTITUTE AND LOCATION
NIAID, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.5	3.0	3.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Using a variety of molecular biological and biochemical techniques, structure/function relationships of several human immunodeficiency virus (HIV) genes have been examined. The role of the "A" (sor) gene product during productive infection was evaluated by deleting a 620 bp segment from an infectious molecular clone of the HIV provirus. This alteration had no effect on viral gene expression or on the asembly or release of reverse transcriptase-containing virions in transfection experiments. However, a 1000-fold reduction in the infectivity of secreted particles was observed. This defect could be restored in transcomplementation experiments using an "A" gene cDNA clone. Efficient transfer of the "A" mutant was observed when virus producing cells were cocultivated with T4+ lymphocytes demonstrating the HIV has the capacity to spread "cell-to-cell" in addition to initiating exogenous infections as cell-free particles.

To investigate the possibility that DNA viruses could augument gene expression of HIV, cotransfection experiments were conducted using the HIV long terminal repeat linked to the chloramphenicol acetyl transferase gene in the presence of DNAs from JCV, BKV, HSV-1, VZV, and bovine papillomavirus. Transactivation was observed with several of these unrelated viral DNAs suggesting that coinfection of cells by HIV and some of these agents could stimulate latent proviruses.

PHS 6040 (Rev 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00433-03 LMM

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Use of Retroviruses as Tagged Insertional Mutagens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Jonathan Silver Medical Officer LMM, NIAID

COOPERATING UNITS *(if any)*

Cindy Edwards, Scientist, LDP, NCI

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Transposable elements have been used in simpler biological systems to clone genes of interest by virtue of the fact that insertion of a transposable element may cause a mutation at the site of integration ("transponson tagging"). To investigate the feasibility of using murine retroviruses as tagged insertional mutagens, I am collaborating with Dr. Cindy Edwards in an attempt to clone the gene for the aryl hydrocarbon (Ah) receptor using a modified Mo-MuLV as the tagged mutagen. Cells containing a single functional copy of the Ah receptor were infected with Mo-MuLV and over 20 mutant cell lines lacking the Ah receptor were selected. We cloned integrated copies of Mo-MuLV along with flanking cellular DNA from these cell lines. Several clones from different cell lines appear to contain a copy of Mo-MuLV integrated in the same fragment of cellular DNA. Further analysis of these cellular DNA fragments is underway.

Significance: These experiments represent an attempt to clone an important gene involved in the metabolism of aryl hydrocarbons, and provide a model system for the use of retroviruses as "tagged transposons" to clone selectable genes in higher eukaryotes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00434-03 LMM

PERIOD COVERED
Period Covered: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Studies on the Dissemination of Streptococcal Antibiotic Resistance Determinants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.J. LeBlanc	Head, BVS,	LMM, NIAID
Others:	L.N. Lee	Chemist	LMM, NIAID

COOPERATING UNITS *(if any)*
Dr. Fred C. Tenover, Seattle Veterans Administration Medical Center, Seattle, WA 98108

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Bacterial Virulence Section

INSTITUTE AND LOCATION
NIAID, NIH, Frederick, Maryland 21701-1013

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Three antibiotic resistance genes, responsible for high-level kanamycin (Km), streptomycin (Sm) and erythromycin (Em) resistance among numerous streptococcal isolates of human and animal origin, may have been disseminated via a common transposon. Streptococcus faecalis strains JH1, LDR175 and LDR517 are all resistant to high levels of Km, Sm and Em. Strain JH1 was isolated from a human clinical patient, strain LDR175 from a healthy chicken, and strain LDR517 was a multiply resistant transconjugant isolate obtained from a mating between strain LDR175 and the plasmid-free recipient strain JH2-2. In strain JH1, the Km, Sm and Em resistance traits are encoded by three distinct genes carried on plasmid pJH1. Each of these genes, cloned from pJH1, hybridized only to chromosomal DNA from strains LDR175 and LDR517. A segment of DNA, >13 kilobase pairs (kbp) in size and carrying all three resistance genes, was transposable to a hemolysin-bacteriocin plasmid, pAD1, when also present in strain LDR517 or a strain harboring pJH1. Preliminary restriction endonuclease maps of the transposed DNA from pJH1 or strain LDR517 appeared identical. Three tetracycline resistance (Tc^R) genes, tetL, tetM, and tetN, have been identified in the genus Streptococcus. We cloned a fourth Tc^R gene, tetO, from a strain of Streptococcus mutans. This cloned gene did not hybridize to tetL, tetM or tetN, but did hybridize, under stringent conditions, to Tc^R plasmids from species of the Gram-negative intestinal pathogen, Campylobacter, and to a cloned Tc^R gene from a strain of Campylobacter jejuni. The Tc^R genes from S. mutans and C. jejuni are being sequenced for further comparative studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00437-03 LMM

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Biology and Genetics of the AIDS Retrovirus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.D. Hoggan	Senior Investigator	LMM, NIAID
Others:	T.A. Folks	Expert	LIR, NIAID
	K. Straubel	Guest Researcher	LMM, NIAID
	S. Schnittman	Staff Fellow	LIR, NIAID
	M. Martin	Lab Chief	LMM, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Continuing studies designed to elucidate the various stages of HIV infection, including virus adsorption, penetration, morphogenesis of new particles and egress from the cell have been carried out using electron-microscopy. Of interest are our EM studies of an HIV mutant in which a major portion of the A gene has been deleted but which is able to produce low levels RT in mass culture. These infected cultures contain a few cells which appear to be dying as they produce large numbers of mature virions while the majority of cells have a few particles on their surface. EM examination of cloned cell populations from such mass cultures demonstrate wide variations in the number and stage of virus particles produced while none of these cultures are able to produce any RT.

Current studies of human macrophages exposed to mycobacterium avian (a frequently occurring opportunistic infection in AIDS patients) clearly show that the cells are capable of ingesting large numbers of bacteria but such bacteria are not killed but continue to multiply until the macrophages literally burst.

Two divergent field strains of HIV (the NY5 and Alabama strains) have been molecularly cloned and are currently being tested for infectivity. Infectious clones will soon be amplified and used for the production of "type" specific polyclonal antibody in various test animals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00438-03 LMM

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Molecular Biology of Cellular Oncogenes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. Santos	Visiting Scientist	LMM, NIAID
Others:	T. Bryan	Microbiologist	LMM, NIAID
	M. Tamame	Visiting Fellow	LMM,NIAID
	T. Alonso	Guest Researcher	LMM,NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Biochemical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	4	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Structural and functional studies of p21 ras proteins. The products of ras genes have essential cellular functions, but nothing is known about their specific functional role and related structural details. We addressed those questions with a combination of approaches: 1) Monoclonal antibodies against p21 proteins. We developed a panel of monoclonal antibodies recognizing every domain of the protein. Correlating the epitope recognized with the effect of each Mab on the biochemical properties of p21 we constructed a functional map of the protein. Also, by using a selected Mab from our panel we identified a related antigen located in mitochondria this may be a useful approach to identifying yet unknown members of the ras gene family. 2) Radiation inactivation of p21 ras proteins. By means of radiation inactivation techniques we determined that the structure of p21 is homooligomeric; this structure clearly differentiates ras proteins from classical G proteins and offers the basis for a model of biochemical action. We propose that activation of p21 involves subunit reorientation within the oligomer upon binding of guanine nucleotides. 3) Signal transduction through the PI pathway. We studied normal and transformed NIH/3T3 cells to assess the effect of ras and other oncogenes in the process of signal transduction. We showed that there are specific alterations in the phosphoinositide pathway in ras transformed cells, but similar changes are produced by many other oncogenes. A specific stimulation of phospholipase A2-type activities and a total uncoupling to signals being transmitted through the PI pathway were observed in all transformed cells studied. These finding are inconsistent with any direct role of p21 proteins as regulatory elements of enzymes of the PI pathway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00464-02 LMM

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Plasmid Replication and Expression in Streptococci

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Jon M. Ranhand Senior Scientist LMM, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Bacterial Virulence Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00466-02 LMM

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Transcriptional Complexity of AIDS Retrovirus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	S. Venkatesan	Expert	LMM, NIAID
Others:	N. Ahmad	Visiting Fellow	LMM, NIAID
	R.J. Mervis	Microbiologist	LMM, NIAID
	E. Lillehoj	Scientist E	FCRC, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In addition to the usual retroviral gag, pol, and env genes, the AIDS retrovirus (HIV) possesses several open reading frames (ORFs) encoded by complex spliced mRNAs. We have concentrated on two of these ORFs, namely the B ORF and the art gene. To determine the functional potential of these transcripts, expressible cDNA plasmids representing viral RNAs were constructed. Complete DNA sequencing of two clones revealed that one of them (pV102) had the 5' leader RNA spliced to a 69 nt central exon which was spliced to a 1300 base 3' exon including the entire B ORF and terminating 12 nucleotides downstream of the poly (A) site in the R sequence of the LTR. The cDNA insert in pV102 lacked the Met codon for the art gene. The other recombinant (pNN37) had intact leader RNA, no central exon and a shorter 3' exon. This clone possessed the entire B ORF but was spliced to the 5' leader 90 bases downstream of the site in pV102.

By cell free translation of the T7 transcripts of the cDNA insert in pV102, it was shown to encode two forms of B protein alternatively initiated at two Met codons separated by 20 residues. The functional role(s) of these protein(s) were determined by constructing infectious proviral DNAs containing either a termination codon after the second Met or a frameshift deletion 100 residues from Met. In both cases, there was minimal effect on transient viral replication compared with the standard provirus. The mutant proviruses replicated indistinguishably from wild type proviral DNA in T lymphocytes. Similarly, overexpression of B gene product did not significantly alter the transient expression wild type or B mutant proviruses. To study the function of the art gene product, pV102 was engineered to supply the initial Met Codon. The resultant plasmid programmed the synthesis of art protein both in vitro and in vivo. It was able to transcomplement a mutant provirus carrying a termination codon in the art ORF. Studies are in progress to try and dissect out the different functions of this protein in DNA transfection experiments.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00467-02 LMM

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure & Function of Polypeptides Encoded by AIDS RV Genomes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	S. Venkatesan	Expert	LMM,	NIAID
Others:	E. Lillehoj	Scientist E	FCRC,	NIAID
	R.J. Mervis	Microbiologist	LMM,	NIAID
	N. Ahmad	Visiting Fellow	LMM,	NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The genome of the AIDS retrovirus (HIV), the etiologic agent of AIDS, codes for several polypeptides in an extremely complex scheme of mRNA biogenesis. We have used kinetic analysis of viral proteins using defined antisera, purified and sequenced several viral proteins both from extracellular virus and cellular lysates. Where possible, we have tried to correlate in vivo protein synthesis with cell free translation of selected viral mRNAs. To study the functional significance of different viral gene products, we have mutagenized the proviral DNA and studied their effects on viral replication using DNA transfections of animal cells. Using a cell line (8E5) containing a single copy of defective proviral DNA with a premature termination codon in the p64 RT domain of the pol ORF, we successfully identified and sequenced the p64 and p51 RT subunits of pol ORF, as well as the p34 endonuclease mapping near the C-terminus of the pol ORF. We have extended this project and successfully identified and sequenced a p10 gag -pol protease responsible for the processing of the gag and gag-pol fusion proteins. The localization of the N-terminus of the gag protease has allowed us to dissect the steps involved in the frameshifting mechanism responsible for the synthesis of gag-pol fusion proteins. We have also developed a sensitive assay for the protease using defined oligopeptides, and this will enable us to screen several protease inhibitors as potential antiviral drugs. During the studies relating to the mechanisms of gag and gag-pol protein processing, we also identified two forms of a gag precursor protein p41 and are presently trying to determine the functional correlation of a p41 protein initiated internally 141 residues from the authentic AUG codon of the gag ORF.

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LVD

LABORATORY OF PARASITIC DISEASES

1987 ANNUAL REPORT

Table of Contents

SUMMARY....................14-1

Project Number

Z01 AI 00094-28 LPD *Entamoeba histolytica*: Nutrition, Differentiation Virulence, Molecular Probes - Diamond....................14-11

Z01 AI 00097-29 LPD Physiological and Cytochemical Pathology of Parasitic Diseases - Mercado....................14-12

Z01 AI 00098-31 LPD Biochemical Mechanisms of Energy Metabolism in Mammalian and Parasitic Organisms - Weinbach....................14-13

Z01 AI 00099-17 LPD Biophysical Parasitology - Dvorak....................14-14

Z01 AI 00102-13 LPD Pathogenesis of Disease Caused by Infection with Intracellular Parasites - Neva....................14-15

Z01 AI 00103-20 LPD Immunological Studies on Toxoplasmosis and Other Parasitic Diseases - Lunde....................14-16

Z01 AI 00108-16 LPD Studies on the Biology and Immunogenicity of Malaria Sporozoites - Gwadz....................14-17

Z01 AI 00161-10 LPD Immunochemistry of Parasitic Diseases - Nash....................14-18

Z01 AI 00162-11 LPD Biochemical Cytology of Host-Parasite Interactions in Parasitic Protozoa - Dwyer....................14-19

Z01 AI 00197-08 LPD Immunoregulation and Immune Recognition in Filariasis and Non-filarial Diseases - Nutman....................14-20

Z01 AI 00208-07 LPD The Isolation and Characterization of Plasmodial Genes - McCutchan....................14-21

Z01 AI 00240-06 LPD Antigenic Analysis of Sexual Stages of Malaria Parasites - Miller/McCutchan....................14-22

Z01 AI 00241-06 LPD Identification of Receptors for Merozoite Invasion of Erythrocytes - Miller....................14-23

Z01 AI 00242-06 LPD Biological and Biochemical Studies of Antigens on Malaria-infected Red Cells - Howard....................14-24

Z01 AI 00244-06 LPD Developmental Adaptations of Trypanosoma cruzi to the Vertebrate Immune System - Sher..............14-25

Z01 AI 00246-05 LPD Molecular Studies of the Genome and Surface of Schistosoma mansoni - Sher..........................14-26

Z01 AI 00248-06 LPD Genetics and Physiology of Vector Capacity in Anopheline Mosquitoes - Gwadz......................14-27

Z01 AI 00251-06 LPD Immunologic Studies on Schistosomiasis - Sher.......14-28

Z01 AI 00253-06 LPD Studies of the Immunologic Responses to Filarial Helminth Infections - Ottesen..............14-29

Z01 AI 00255-06 LPD Studies of the Immunologic Responses to Non-filarial Helminth Infections - Ottesen..........14-30

Z01 AI 00256-06 LPD Differentiation of Leishmania Promastigotes - Sacks..............................14-31

Z01 AI 00257-06 LPD Immunology of Strongyloidiasis - Neva...............14-32

Z01 AI 00347-05 LPD Studies on the Schistosomal Hepatic Fibrosis - Cheever..........................14-33

Z01 AI 00348-04 LPD Studies on the Immunity in Murine Schistosomiasis - Cheever..................14-34

Z01 AI 00350-05 LPD DNA Analysis of Parasites - Nash...................14-35

Z01 AI 00351-05 LPD Parasite and Host Factors Controlling the Pathogenesis of Leishmaniasis - Scott...............14-36

Z01 AI 00439-03 LPD Clinical and Therapeutic Studies in Human Filariasis - Ottesen...............................14-37

Z01 AI 00483-02 LPD Molecular Genetics of Malaria - Wellems............14-38

Z01 AI 00487-01 LPD Studies on the Quantitative Parasitology of Schistosome Infections - Cheever..................14-39

Z01 AI 00494-01 LPD Analysis of T Cell Responses in Human Leishmaniasis - Sacks.............................14-40

Laboratory of Parasitic Diseases

National Institute of Allergy and Infectious Diseases

Summary - October 1, 1986 - September 30, 1987

ADMINISTRATIVE AND ORGANIZATIONAL EVENTS

Most of the administrative preoccupation for LPD in the last year has been with the future move scheduled for the summer of 1988 to the renovated Building 4 -- currently a jumble of construction to the west. We seemed to have learned to live with the new FTE system and have found that we can survive. The decentralized budget system, which seemed so formidable initially, also seems to be working out and we are grateful for funding in the past year, especially to replace badly needed equipment.

The following professional personnel have departed for other jobs or other activities: we will sorely miss Dr. Richard Carter who decided to go back to the United Kingdom as a British Medical Research Council/Scientist in Edinburgh, Scotland, but we intend to continue our collaboration with him in malaria research. Two departing Medical Staff Fellows were James Sherwood and Richard Finley; the latter left for a temporary assignment in Brazil. Postdoctoral Fellows David Lanar and Frank Klotz left to take positions with the Walter Reed Army Institute of Research along with James Sherwood -- we seem to provide many of their research personnel! Douglas Ward, another Medical Staff Fellow, will be in the Washington area as an infectious disease clinician. Alan Saul, who was with us as a Visiting Scientist, returned to his job in Australia and Ilona Bonsch, a predoctoral fellow, returned to Germany to get her doctoral degree. Two long-time LPD research assistants retired, Bruce Hill and Rodney Duvall. The absence of Rodney Duvall, who assumed so many LPD responsibilities and was so helpful to many incoming young postdoctoral research fellows, will be especially missed.

Incoming professional personnel include the following: there are two Medical Staff Fellows, Dr. Michael Ballard and Dr. Alimuddin Zumla, the latter of whom will spend most of his first year in LCI before coming to LPD for his research. Andrew Waters, who had been here for a short period with the malaria group from England, returns as a Visiting Associate. Several new Visiting Fellows include Dr. John Dalton, Sanjai Kumar and Jong-Youn Lee, all of whom will be working with the Malaria Section. Melanie Small is a Guest Worker from Georgetown University working with the malaria group. The Bhattacharyas, Alok and Sudha, are back working with Dr. Diamond for a short term and will continue to collaborate with him. We were fortunate in having an experienced secretary, Jeanne Carolan, join our office staff and more recently, Geraldine Carter, who filled a position left by the departing Betty McChesney on our office staff.

The travel of LPD staff to attend WHO meetings in Geneva or workshops and other meetings abroad are not enumerated here. However, travel abroad for research work by LPD staff included Drs. Sacks and Neva in Patna, India for work on kala-azar, Drs. Nutman, Freedman and Ottesen in Madras, India on filariasis and Dr. Ottesen will be in Guatemala in September studying patients with visual loss caused by onchocerciasis. In addition, Drs. Michael Good and Isabella Quakyi along with Howard Hughes fellow David Pombo spent about 6 weeks at the Medical Research Council Laboratory in Gambia working on malaria. Although not in the category of foreign travel, Dr. Russell Howard has been on special assignment at the laboratory of former fellow James Leech at the University of California and also doing some collaborative work with scientists at DNAX.

HONORS AND AWARDS

Many of the LPD staff contribute to the administrative functioning of the NIH, NIAID, and even at the level of the Laboratory by serving on Promotion and Tenure Committees, Library Committees, Safety Committee, etc. -- as well as a number of Ad Hoc Committees of the Institute organized to address specific issues such as computer use and data processing.

In addition to the above, many of the professional staff serve on WHO Expert Committees and Steering Committees and review research grants for various granting agencies. They also are invited participants to meetings or workshops, and spend a great deal of time reviewing manuscripts. Such recognition and honors are too numerous to be mentioned individually.

The following items, therefore, can be considered to merit special mention:

Dr. Dennis Dwyer was a recipient of a NIH Director's Award.

Mr. Milford Lunde received a special NIH Merit Award.

Drs. David Sacks and Alan Sher have been appointed as section editors for the Journal of Immunology. In addition, Dr. Sher continued his third summer as co-director of the Biology of Parasitism Course, at the Marine Biological Lab, Woods Hole, MA.

Dr. Diamond was a 1986 recipient of the Anniversary Award of the Helminthogical Society of Washington.

Dr. Lewis Miller was asked to serve as Chairman of the Burrough's-Wellcome Special Research Fellowship Committee on Parasitology.

Dr. Neva was the invited Craig Lecturer for the 1986 Annual Meeting of the American Society of Tropical Medicine and Hygiene.

RESEARCH PROGRESS

MOLECULAR ANALYSIS OF MALARIAL VACCINES

Limitations of recombinant and synthetic peptide antigens. Last year's report commented upon the importance of T-cell recognition sites in the recombinant sporozoite vaccine as revealed by genetic control of immune response using in-bred strains of mice (Good *et al*.). This concept was examined further in a rodent system with *P. yoelii* which showed that immunization with synthetic peptides failed to protect mice against sporozoite induced infection even though antibodies against sporozoites were produced (A. Lal, de la Cruz, Good, Weiss, *et al*.).

Final results on first trial of recombinant sporozoite vaccine FSV-1 in humans. The phase I trial of recombinant sporozoite *P. falciparum* vaccine done in collaboration with WRAIR was extended to include a fourth dose one year after the third dose. Three weeks after the final dose, six vaccine recipients and two unimmunized controls were exposed to infected mosquitoes. Only one of the six challenged individuals was protected; all other exposed subjects developed malaria. Although this trial demonstrated that a recombinant sporozoite vaccine could protect against human falciparum malaria, the vaccine failed to produce significant booster responses and the efficacy was unacceptably low (Sherwood and Neva of LPD, and numerous WRAIR collaborators).

Analysis of T-cell recognition sites of an antigen in humans. In order to determine the specific domains on a molecule recognized by T-cells, a series of 29 synthetic overlapping peptides covering the immunodominant region of the *P. falciparum* sporozoite protein were prepared. They were then tested *in vitro* for blastogenic activity with lymphocytes from patients, presumably immune, in a malaria endemic area of the Gambia. Blastogenic responses were found to occur primarily against a relatively restricted site of the CS protein molecule. (Good, Quakyi, Miller and Pombo of LPD; Alling of NIAID; Riley and Menon of MRC Lab, Gambia; Berzofsky of NCI and Houghten of Scripps, CA).

An artificial antigen containing both B and T-cell epitopes. Since the ultimate effector mechanism(s) for effective anti-sporozoite immunity are still not early established, a combination of T- and B-cell immunity may be desirable. Toward this end, an artificial synthetic antigen was made by covalent linkage of a T-cell site with a major B-cell site on the molecule of the CS protein of *P. falciparum*. This work was done by using an algorithm designed to predict T-cell sites and a large panel of H-2 congenic mice (Good, Lunde and Miller of LPD; Malloy of LIG; Smith and Moss of LVD and Nargalit, Cornette and Berzofsky of NCI).

OTHER APPROACHES IN MALARIA RESEARCH NEEDED

Recent disappointments with the recombinant vaccine in light of increasing problems associated with malaria emphasize the need for a broad base of new approaches in malaria research. Some are indicated here.

Molecular genetics to map chromosomes of the malaria parasite. By pulsed field gradient gel electrophoresis, chromosome mapping and genetic crossing is being used to investigate linkage groups and to identify genes associated with specific markers. One end of chromosome 13 was found to contain a gene affecting asexual stage proliferation of *P. falciparum in vitro*. A genetic cross between a multi-drug resistant and a drug sensitive clone is underway and progeny will be examined for inheritance of resistance and evidence of linkage (Wellems, Panton, Sinnis, Gwadz of LPD and outside collaborators).

No new approaches to possible control of Anopheline malaria vectors have been developed since introduction of residual insecticides over 40 years ago. After long and tedious efforts to work out techniques, introduction of a foreign gene into *Anopheles gambiae* eggs was successfully accomplished (Miller, Sakai, Romans of LPD and Coon of NCI). The mechanism of encapsulation which renders certain *Anopheline* species resistant to malaria parasite development is believed to be under genetic control. In any event, transfer of genetic material into *Anophelines* may permit new methods of manipulating this important disease vector.

Probes for ribosomal RNA found to *be unique and more sensitive than probes for DNA*. Most work dealing with molecular probes for detection of small numbers of microorganisms utilize DNA probes. With a *P. berhei* system ribosomal RNA probes were found to be several orders of magnitude more sensitive than DNA probes. Also, ribosomal RNA was found to be expressed differently in the invertebrate host as compared to the vertebrate host (McCutchan of LPD).

Parasite ligands identified that bind to red cell receptors. Under special culture conditions with only few uninfected red cells, a protein from *P. knowlesi* merozoites that normally binds to red cells accumulates in the culture fluid. This 135 kDa ligand binds to all Duffy positive red cells but not to Duffy negative red cells. On the basis of ability of certain sulfated sugars with heparin binding sites to block binding of the 135 kDa ligand to Duffy receptors, the Duffy blood group antigen is believed to be a sulfated proteoglycan (Miller and Dalton of LPD and Haynes of WRAIR). *P. falciparum* parasites were found to be capable of invading mouse erythrocytes, although they do not develop after invasion. This apparent oddity may not be so strange when the previous observation of McCutchan is recalled that on a molecular basis, *P. falciparum* more closely resembles avian and rodent malarias than other primate malaria parasites (Miller and Klotz of LPD).

Further progress on analysis of antigens of sexual stages of malaria parasites. Immunization with selected antigens of the sexual stages of the malaria parasite (gametes and ookinetes) to produce transmission-blocking immunity is still a feasible objective and work on this continues. One technical advance has been improvements in the large-scale automated *in vitro* culture of gametocytes using synthetic serum. Over 10^9 *P. gallinaceum* and *P. falciparum* zygotes have been accumulated for use in microsequencing target antigens. Already several oligonucleotide probes have been constructed which hybridize to restriction fragments of the genome to indicate that it should be possible to clone the genes which make the proteins of interest (Miller, McCutchan, Kaslow, Syin and Quakyi of LPD, Coligan of LIG and Carter in the U.K.).

TRYPANOSOMA CRUZI

Population dynamics of parasite clone mixtures grown in culture vs. in the vector bug. As reported last year, faster growing clones of *T. cruzi* rapidly replace clones with a slower rate of growth in mixed culture. It was found that mixtures of clones in the vector bug co-exist for a longer period of time, but the faster grower eventually replaces the slower growing population. In a computer-simulated experiment of clone mixtures grown in vertebrate cells, it appears that some mixtures will persist independently, but this has not yet been tested in cell cultures. The bottom line message from these experiments is to isolate single cell clones as early as possible from clinical specimens if characteristics of parasite strains are to be evaluated (Finley and Dvorak of LPD).

Electrocardiography in genetic in-bred strains of mice chronically infected with different clones of T. cruzi. By electronic "slow motion," a method for obtaining electrocardiograms on anesthetized mice was developed. Interestingly, the normal ECG pattern was found to differ significantly between C3H and C57 mice. Virulent clones of *T. cruzi* produced many of the ECG changes in mice after 1 year of infection as seen in humans with chronic Chagas' heart disease (Dvorak of LPD and Bailey of DCRT).

Electron beam X-ray microelemental image analysis of T. cruzi. A marked difference in colors between clones of *T. cruzi* grown in liquid media was found to reflect great differences in certain elemental constituents, especially Fe and Zn. These differences are a consequence of active transport of the various elements from the growth medium into the parasite. A marked increase in cytochrome content of the clones was also found (Dvorak, Engel of LPD and Schuette).

Interaction of T. cruzi with complement. Further progress has been made in characterizing the factor produced in *T. cruzi* culture supernates which accelerates the decay of complement (actually C3 convertase). The factor is 80-90 K in size, is heat labile, protease sensitive, and absorbed by Con-A sepharose - characteristics which make it indistinguishable from human DAF. A cDNA library has been constructed for attempts to clone the molecule (Sher of LPD and Joiner and Grimaldi of LCI).

Definition of stage-specific gene products in metacyclic trypanosomes. Since metacyclic tryps are the infective stage for vertebrate cells, finding gene products unique to this stage of the parasite is desirable. Translation products of mRNA produced from trypomastigotes and epimastigotes were immunoprecipitated with antisera raised vs. trypomastigote antigens. A 66 K band was precipitated from the translation products indicating that antigenic polypeptides specific for trypomastigotes are among the gene products (Heath and Sher of LPD).

Anti-trypanosomal factor (ATF) from Pseudomonas species. Countercurrent chromatography has proved to be the most effective method for fractionation of the material from cultures of Pseudomonas fluorescens that kills trypanosomes. Two types of compounds have been separated -- one that lyses the parasites, the other imobilizes and kills but does not lyse. Sugar and amino acid analyses have shown presence of mannose and several aliphatic and aromatic amino acids. Even though purification of the ATF has been improved, its properties make this task difficult (Mercado of LPD and Rice of NIADDK).

IMMUNE RESPONSE AND TREATMENT OF FILARIAL INFECTIONS

Associations of IgG subclass response with clinical manifestations of filariasis. As reported last year, patients with asymptomatic filarial infections who often exhibit "blocking antibodies" that inhibit antigen-induced, IgE mediated histamine release in vitro, also have elevated IgG4 levels. Attempts to remove the IgG4 fractions by use of affinity columns containing anti-subclass monoclonal antibodies in order to test for blocking activity gave equivocal results. So, the subclass identity of blocking antibodies remains in doubt. However, patients with chronic lymphatic obstructive pathology were found to have striking IgG3 antibody levels. The reasons for these differences in antibody response must somehow relate to the pathology of the disease (Hussain and Ottesen of LPD).

Identification of T-cell subsets collected and transported from the field. Since many of our immunologic studies are done on patients abroad there is a great need for defining conditions by which clinical specimens can be collected in the field and transported to the NIH for studies that require more complex instrumentation. A panel of reagents for identifying T-cells subsets by flow cytometry was tested on cells from patients and controls in India. It was found that mononuclear cells labeled with the reagents, fixed and transported back to the NIH could be reliably sorted up to 2 weeks later (Lal, Nutman and Ottesen of LPD).

Immunopathology of ocular onchocerciasis. Several types of ocular tissues (conjunctiva, iris and lens) as well as aqueous humor were obtained in the course of cataract surgery from 22 Ghanian patients with and without onchocerciasis. By staining with reagents for T-cell subsets, infiltration of suppressor T-lymphocytes in the conjunctiva and iris of onchocerciasis patients was significantly greater than in controls. Helper T-cell preponderance is generally found in most cases of non-parasitic uveitis (Chan and Nussenblatt of NEI and Ward and Ottesen of LCI). Anti-onchocera IgG antibody was found in the aqueous of onchocerca patients in amounts suggesting local production or accumulation of this specific antibody in the ocular compartment. Anti-retinal autoantibodies were prominent in the serum and aqueous of the patients but not in the controls (Ottesen of LPD and Chan and Nussenblatt of NEI).

Anti-eosinophil reagents developed to study the kinetics and effector functions of eosinophils in parasitic disorders. Four prominent eosinophil molecules [major basic protein (MBP), eosinophil peroxidase (EPO), eosinophil derived neurotoxin (EDN), and eosinophil cationic protein (ECP)] have been chromatographically purified from cells of patients with various hypereosinophilic disorders.

Polyclonal antisera from rabbits against all four proteins and two monoclonal antibodies to MBP and EPO have also been developed. These reagents will obviously be useful to identify and quantify the various eosinophil constituents in human specimens (Davey, Nutman and Ottesen of LPD).

Treatment of lymphatic filariasis with the new anti-helminthic, Ivermectin. The new anti-nematode drug, Ivermectin, which has been used up to now mainly for onchocerciasis, was tested for efficacy in microfilaremic patients with W. *bancrofti* in Madras, India. In the first study groups of 10 patients each were treated with single daily doses ranging from 15 to 200 mcg/kg. Microfilaremia cleared with all doses by 2 weeks, but returned to 30 percent of pre-treatment levels by 6 months. Side effects were common with severity related to degree of microfilaremia, but tending to be less at the lowest dose level. In a second trial Ivermectin was compared with DEC in a blinded, placebo controlled trial. The efficacy of the two drugs was approximately equal although late follow-up return of microfilaremia is not yet available. In view of the fact that a single dose of Ivermectin was just as effective as 13 days of DEC, the former drug was clearly superior (Ottesen and Nutman of LPD; Prabhakar, Vijayasekaran and Kumaraswami of ICMR, Madras and Merck, Sharp and Dohme).

MOLECULAR BIOLOGY OF SCHISTOSOMES

The paramyosin antigen in schistosomes needs some molecular engineering. As reported last year one of the major immunogens in *S. mansoni* was found to be an invertebrate muscle protein, paramyosin. The cDNA for this product that was cloned, comprising about 60 percent of the gene, appears to lack crucial epitopes required to elicit protection as an antigen. Presence of introns in several additional clones that were identified will mean some complications in sequencing the entire gene, but an improved antigenic gene product will be sought. cDNA clones of paramyosin have also been identified from *S. japonicum*. These will be compared to the *S. mansoni* product (Lanar, Pearce, Sher of LPD and Donelson of University of Iowa).

Molecular marker for drug resistance in schistosomes. The story of the marker for hycanthone resistance in schistosomes described in last year's report still holds up, but it has become more complicated. Some drug resistant progeny may not express the characteristic 3.8 Kb marker fragment in their DNA, but they do show a characteristic pattern of restriction fragment length polymorphism. Also, differences between drug sensitive and resistant worms can be seen in two-dimensional SDS-PAGE patterns of mRNA translation products (Brindley and Sher of LPD and Lewis of Biomed Research Institute).

IMMUNOLOGY AND EXPERIMENTAL PATHOLOGY OF SCHISTOSOMIASIS

Control of complement activation by schistosomes is limited to autologous host species. It has long been known that cercariae and early developmental schistosomulae activate the alternate complement pathway (ACP), whereas even young worms and adults fail to do so. It was recently found that this regulation of the ACP was restricted to complement from the homologous species. Thus, human and rabbit complement, for example, would be activated by schistosomes from guinea pigs. Exposure of worms to certain enzymes, such as neuraminidase or phospholipase C, will convert the parasite to an activator of autologous complement. These findings suggest that the schistosome membrane acquires species specific regulatory molecules from the host which control activation of autologous complement (Pearce and Sher of LPD and Hall of LCI).

Immunologic manipulation of murine hosts to influence hepatic granulomas and fibrosis. Clearly T-cells are required for formation of schistosome egg granulomas so infected nude mice were given different subsets (L3T4 or Ly 2.2) of T-cells to see what would happen to their egg granulomas. Although normal size granulomas were restored around *S. japonicum* eggs after receipt of spleen cells, granuloma size was equally effected by L3T4 and Ly 2.2 subsets of T-cells. Nudes reconstituted with L3T4 showed many eosinophils in egg granulomas and moderate hepatic fibrosis in contrast to Ly 2.2 restoration. In contrast to *S. mansoni* infected mice in which eggs persist in the tissues for a relatively short time (half-life of 4 weeks), *S. japonicum* eggs persist for at least twice as long. If prolonged persistance of *S. japonicum* eggs in the tissues occurs in humans, then doing egg counts at autopsy to relate findings to pathology is meaningful (Cheever of LPD and Malley of DCRT).

IMMUNOREGULATION AND IMMUNE RECOGNITION IN HELMINTH INFECTIONS

T-cell responses in patients with filarial infections. Certain subsets of patients with filarial infections (such as some types of leishmaniasis or leprosy) can exhibit variable manifestations of antigen specific T-cell unresponsiveness. In the case of microfilaremic patients, evidence for a genetic basis is being sought using HLA and T-cell receptor markers. Some patients with active onchocerciasis were also found to exhibit T-cell unresponsiveness by failure of IL2 production (Nutman, Ward and Ottesen of LPD). In an effort to characterize more precisely the T-cell response to filarial parasites, a study of responses to cloned antigens has been initiated. Two recombinant antigens, one of which has been sequenced, have been identified which stimulate T cells to proliferate. In addition, a number of filarial antigen-specific T cell clones have been established (Nutman, Ward and Davey of LPD).

B-cell responses are being studied with the aid of virus transformed T or B cells. In an effort to better understand immunoregulation of IgE antibody production, T cell clones transformed by HTLV-1 virus were prepared from patients with certain filarial infections and with hyper-IgE syndrome, conditions characterized by elevated IgE antibody levels. Some of these T cell clones are positive for Fc epsilon receptor and make large quantities of IgE binding factors, which in turn can selectively regulate IgE response in vitro (Nutman of LPD; Volkman of Stony Brook, NY and Delepesse of the Univ. of Winnipeg, Canada). Clones of B cells, transformed by EB virus, have been established from patients with filarial infections and onchocerciasis. Some of these clones produce parasite specific antibodies, and the parasite antigens recognized by these B cell clones are being identified by immunoblotting analysis (Nutman and Ottesen). Considerable cross-reactivity of sera from patients with different filarial infections (e.g., loiasis vs. onchocerciasis) is seen when reacted by immunoblotting with a given filarial antigen. However, characteristic patterns are being recognized, which along with the reactions obtained with monoclonal antibodies, give promise of identifying antigens specific for each parasite (Davey and Nutman). Soluble egg antigen (SEA) of S. mansoni was found to induce human endothelial cell proliferation in a dose-dependent manner, at concentrations as low as 30 ng/ml. However, SEA from S. hematobium or S. mekongi, even at concentrations as high as 3000 ng/ml, failed to induce proliferation of cells. This endothelial growth factor effect of schistosome egg antigen may relate to some of the revascularization seen in certain types of pathology of schistosomiasis (Freedman and Ottesen of LPD).

MOLECULAR BIOLOGY AND BIOCHEMISTRY OF GIARDIA AND AMEBAE

Antigenic variation in Giardia. The surface antigens of cloned populations of Giardia were found to vary. Cloned populations succesively to a series of different cytotoxic MAbs allowed the survival of progeny whose surface antigen(s) differed from the previous parental clones. Frequent rearrangement of part of the genome encoding for one surface antigen was common. The same surface antigen was cysteine rich and contained a repeating peptide. Other varying antigens of this and other isolates contained a large amount of cysteine. Preliminary evidence suggest Giardia vary in vivo in animals models and humans.

Studies with E. histolytica. Highly repeated DNA of E. histolytica clones in a plasmid vector was shown to code for ribosomal RNA. Moreover, subfragments generated by TAq1 restriction endonuclease yielded a pattern of fragments that permitted differentiation of E. histolytica from seven other related species of the genus (S. Bhattacharya and Diamond of LPD). The first available serum-free medium (PD-756) for large scale axenic growth of E. histolytica will permit high specificity radiolabelling of the organism and identification of its nutritional requirements (Diamond of LPD).

LEISHMANIAL BIOCHEMISTRY

Further characterization of the two surface membrane nucleotidase of L. donovani. The 3' nucleotidase was purified and shown to be a 43 kDa mannosylated glycoprotein. Antibodies to the enzyme are being used to screen cDNA expression libraries to identify and clone the gene. The 5' nucleotidase was also shown to be a mannosylated glycoprotein containing two subunits of 72 and 43 kDa (Pastakia and Dwyer). The biosynthetic secretory pathway of the soluble acid phosphatase (S-AcP) was localized in both promatigotes and amastigotes of *L. donovani*. The first 30 residues of its N-terminal end amino acid sequence was determined, and oligonucleotide probes based on this sequence were used to identify and clone genomic DNA fragments for the S-AcP gene (Bates and Dwyer of LPD and Gottlieb of Johns Hopkins).

IMMUNOLOGY OF LEISHMANIAL INFECTIONS

Progress on isolation and characterization of a protective antigen of leishmania. Multiple experiments have now confirmed that an excellent degree of protection in mice can be obtained with a soluble extract from *L. major* that can be recovered from a specific fraction (fraction 9) initially separated by FPLC. This fraction contains six relatively small (<25,000) molecular weight proteins. Protection in mice correlates with cell-mediated immunity. Candidate antigens are being purified further to assess protective capacity, as well as to raise monospecific and monoclonal antibodies for use as reagents (Scott, Pearce, Heath and Sher of LPD). T-cell clones have been produced from mice immunized with fraction 9. These will be used in cell transfer studies in mice to test for protective ability and in special immunoblotting tests to check which antigens are recognized by the T cell clones (Scott, Sher and Pearce).

Application of a T cell immunoblotting technique to study cell mediated immune responses in patients. A technique was developed by which soluble leishmanial antigens were first separated by SDS-PAGE, then blotted onto nitrocellulose paper, then cut and transferred to individual wells of a microtiter plate to be cultured with lymphocytes. In this way different antigen fractions can be tested directly for their ability to stimulate lymphocyte proliferation. The technique has been used to study lymphocyte proliferative responses of patients with cutaneous and mucosal leishmaniasis, as well as an individual with healed cutaneous disease caused by *L. donovani*. T cell clones derived from a patient with mucocutaneous disease are also being analyzed (Melby, Sacks and Neva of LPD).

Diffuse cutaneous leishmaniasis in Mexico. At least six newly recognized cases of diffuse cutaneous leishmaniasis (DCL) have been identified in several distinctly different geographic areas of the country. In one location the occurrence of regular cutaneous leishmaniasis is also quite prevalent. The recognition of so many instances of what has been considered a rare complication of cutaneous leishmaniasis is very unusual. The parasite isolates are being typed and characterized (Neva, Savarino of Duke University, and Velasco of Mexico).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00094-28 LPD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Entamoeba histolytica: Nutrition, Differentiation, Virulence, Molecular Probes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L.S. Diamond	Section Head	LPD, NIAID
Others:	A. Bhattacharya	Volunteer Worker	LPD, NIAID
	S. Bhattacharya	Volunteer Worker	LPD, NIAID
	E.C. Weinbach	Section Head	LPD, NIAID

COOPERATING UNITS *(if any)*

Department of Biophysics, Weizmann Institute of Science (D. Mirelman) American Type Culture Collection (P.-M. Daggett, T. Nerad)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Growth and Differentiation

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 2.9	PROFESSIONAL: 1.6	OTHER: 1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A serum-free, partly defined medium has been devised for the axenic cultivation of *Entamoeba* including the human pathogen *E. histolytica*, and the reptilian pathogen *E. invadens*. A critical component is a heat stable, low-molecular weight, growth factor(s) isolated from casein.

Highly repeated DNA of *E. histolytica* was cloned in a plasmid vector and shown to code for ribosomal RNA by its strong homology with *Plasmodium berghei* ribosomal DNA and by Northern blot analysis. Subfragments of the highly repeated DNA generated by restriction enzyme treatment were highly specific for *E. histolytica* hybridizing with DNA from several strains of this species, but not with any of seven other species of *Entamoeba* tested.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00097-29 LPD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Physiological and Cytochemical Pathology of Parasitic Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.I. Mercado	Research Physiologist	LPD, NIAID
Others:	V.J. Ferrans	Chief, Ultrastructure Section	PB, NHLBI
	Y. Ito	Medical Officer	IR, TD, NHLBI
	H.D. Hochstein	Chief, Biological Testing and Reference Standards Branch	DCA, BOB
	K.C. Rice	Research Chemist	LC, NIDDK
	W.G. Coleman, Jr.	Research Microbiolgist	LBP, NIDDK

COOPERATING UNITS *(if any)*

Waters Life Science Application Laboratory, Faifax, VA (M.P. Strickler)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Physiology and Biochemistry

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies on an anti-trypanosomal factor (ATF) from the bacterial species, Pseudomonas fluorescens disclosed that countercurrent chromatography (CCC) employing the Ito multilayer coiled column was very effective for the fractionation of larger samples of the crude product without decreasing resolution or prolonging the speed of separation. Significant lytic activity was produced by amounts of the purified fractions as low as 80 µg. It was also possible to separate two types of components, one that lysed the parasites and one that paralysed and killed, but did not lyse. The difference between these factors was established further on the basis of protein assays and ultrastructural studies of trypomastigotes of Trypanosoma equiperdum treated with these fractions, and with high performance liquid chromatography (HPLC). The CCC-HPLC system will aid in future research on the structure and synthesis of this antibiotic and its chemotherapeutic effect in experimental infections with T. cruzi.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00098-31 LPD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biochemical Mechanisms of Energy Metabolism in Mammalian and Parasitic Organisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	E.C. Weinbach	Section Head	LPD, NIAID
Others:	P. Doyle	Visiting Fellow	LPD, NIAID
	C.E. Claggett	Bio. Lab. Tech. (Chemist)	LPD, NIAID
	L. Diamond	Research Zoologist	LPD, NIAID
	L. Levenbook	Research Chemist	LPB, NIADDK

COOPERATING UNITS *(if any)*
Ciba Pharmaceutical Company (J.L. Costa)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Physiology and Biochemistry

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	2	2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies to elucidate the function of calmodulin (CaM) in Giardia lamblia focused on calcium transport by the intact trophozoites and ATPase activity. CA2+ uptake by the parasite was both rapid and substantial: 224 ± 73 nmoles Ca^{2+}/mg protein/min. The uptake is an active process and appears to be mediated by a membrane-bound Ca^{2+} activated ATPase. Both the Ca^{2+} uptake and the particulate ATPase were inhibited by micromolar concentrations of the CaM antagonists fluphenazine and trifluoperazine. Studies of the effects of tricyclic antidepressant drugs on the in vitro growth of Trypanosoma cruzi showed that chlorimipramine and 2-nitrodesmethylimipramine were cidal to the epimastigotes in micromolar concentations. Differences in susceptibility and development of drug resistance were evident in various T. cruzi stocks. Continued work on the novel Mn^{2+} activated NADPH dehydrogenase provided new evidence that this enzyme may be a specific biochemical marker for the Entamoeba. A survey of the respiratory metabolism of Entamoeba invadens disclosed that this pathogenic parasite has similar properties of electron transport, sensitivity to inhibitors, and respiratory rate as trophozoites of E. histolytica. Mammalian studies were concluded on the effects of tricyclic antidepressant drugs on energy metabolism of human platelets. Cogeners of imipramine that were most effective in suppressing metabolism of platelets were similarly effective in other biological systems (liver and heart mitochndria, chromaffin granules) suggesting a common mode of action, presumably at the membrane locus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00099-17 LPD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Biophysical Parasitology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.A. Dvorak	Res. Microbiologist,	LPD, NIAID
Others:	W. Schuette	Section Chief	ACE, BEIB
	J.J. Bailey		LAS, DCRT
	J.C. Engel	Fogarty Fellow	LPD, NIAID
	R.W. Finley	Med. Staff Fellow	LPD, NIAID
	J. Kwon-Chung	Res. Microbiologist	LCI, NIAID
	R. Leapman	Res. Assoc.	BEIB

COOPERATING UNITS (if any)

Biomedical Engineering and Instrumentation; Branch, DRS; Laboratory of Applied Studies, DCRT; Laboratory of Clinical Investigation

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is concerned with (a) the genetic diversity of Trypanosoma cruzi and its implications to the epidemiology, course and diagnosis of Chagas' disease, (b) the development of high resolution flow cytometry instrumentation for analyses of cell populations and, (c) the utilization of flow cytometry and low light level video microscopy for the analyes of infectious agents. Flow cytometry was used to analyze the population dynamics of T. cruzi clone mixtures. The relative numbers of each clone in the population changed rapidly and the results are in quantitative agreement with mathematical models of competitive population growth; no dynamic equilibrium was found. These data emphasize the importance of working with well-defined clones in the laboratory and stress the importance of rapid isolation of single clones from clinical specimens. A similar situation occurs in Dipetalogaster maximus except that clones can coexist for longer periods of time in the bug. Electrocardiographic analyses of inbred mice infected with T. cruzi clones demonstrate that several known aspects of chronic Chagas' disease can be mimiced with the model. Electron beam x-ray microchemical analyses of T. cruzi clones demonstrate marked inter-clonal differences in Fe, Zn, S, Mg, K, Ca and P.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00102-13 LPD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pathogenesis of Disease Caused by Infection with Intracellular Parasites

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	F.A. Neva	Chief	LPD, NIAID
Others:	D. Sacks	Research Microbiologist	LPD, NIAID
	P.A. Scott	Expert	LPD, NIAID
	J.A. Sherwood	Med. Staff Fellow	LPD, NIAID
	S. James	Senior Investigator	LCI, NIAID
	J. Ruthlein	Guest Researcher	LCI, NIAID

COOPERATING UNITS *(if any)* Univ. of Arizona, Tucson (E.A. Petersen), Harvard Med. School, Boston, MA (F. von Lichtenberg); Inst. of Dermatology, Santa Domingo, Dominican Republic (H. Bogaert); Institut de Salubridad y Enfermadades Tropicales, Mexico City (O. Velasco); Rajendra Memorial Inst., Patna, India (A.B. Sen and staff);

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Cell Biology and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Work on leishmaniasis again dominated most of the effort on this project. An initiative on locating new cases of diffuse cutaneous leishmaniasis (DCL) in Mexico was successful. Six cases of advanced DCL were found in at least 3 different geographic areas of Mexico, where this variety of leishmaniasis has been reported in the past but not emphasized recently. Some of the DCL isolates have been typed as members of the L. mexicana complex. Eight additional new cases of cutaneous leishmaniasis and one new case of mucocutaneous disease, from diverse geographic areas, were diagnosed, evaluated immunologically, and most were treated at NIH. We still have not been able to identify more precisely the immuno-suppressive population of lymphocytes in DCL patients. Experiments with frozen lymphocytes from DCL patients were found to sometimes yield results at variance with those obtained with fresh cells.

The collaborative study with WRAIR on the efficacy of a recombinant sporozoite vaccine against P. falciparum in human volunteers was completed. One of six volunteers immunized with 4 doses of the experimental vaccine was protected when challenged with infected mosquitoes three weeks after the last dose of vaccine. Although the vaccine was protective in one individual, the lack of booster response to multiple doses, and re-evaluation of how anti-sporozoite immunity is produced indicate that more basic studies of this type of vaccine for humans are needed.

LMSF
LPB
LPVD
RMOB
LVD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 A1 00103-20 LPD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunological Studies on Toxoplasmosis and Other Parasitic Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Milford N. Lunde		
Others:	A. W. Cheever	Assistant Chief	LPD, NIAID
	T. E. Nash	Senior Investigator	LPD, NIAID
	E. A. Ottesen	Senior Investigator	LPD, NIAID
	Leon Jacobs	Scientists Emeritius	NIAID
	Henry Masur	Deputy Chief	CCM, CC
	J. P. Dubey	Microbiologist	USDA, API. Beltsville, MD

COOPERATING UNITS *(if any)*

U.S. Department of Agriculture, Animal Parasitology Institute, Dr. John Cross, USUHS and NAMRU 2 Manila, Philippines

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Host-Parasite Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	0.9	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Toxoplasmosis. An experiment is in progress to determine if reactivation of toxoplasmosis in immunocompromised hosts is due to proliferation of "cryptic" endozoites or cyst rupture or both. Studies show that trimetrexate combined with sulfadiazine in the treatment of toxoplasmosis may be a safe and effective alternative to pyrimethamine and sulfadiazine in the treatment of toxoplasmosis. Circulating antigen has been found in sheep infected with toxoplasmosis beginning 2 weeks post infection. Antigen has also been found in the aborted fetuses from acutely infected sheep.

Schistosomiasis. ELISA for the detection of circulating antigen (GASP) reported last year is still being evaluated. Sera are currently being examined from *S. japonicum* patients from NAMRU 2 in the Philippines.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00108-16 LPD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on the Biology and Immunogenicity of Malaria Sporozoites

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.W. Gwadz	Senior Scientist	LPD, NIAID
	M.F. Good	Guest Worker	LPD, NIAID
Others:	T. McCutchan	Senior Scientist	LPD, NIAID
	W. Weiss	Guest Worker	LPD, NIAID
	D. Pombo	Guest Worker	LPD, NIAID
	M.L. Maloy	Senior Scientist	LIC, NIAID
	I.A. Quakyi	Guest Worker	LPD, NIAID
	L.H. Miller	Head, Malaria Section	LPD, NIAID

COOPERATING UNITS *(if any)*

J.A. Berzofsky, Senior Scientist, Metabolism Branch, NCI; Ann Szarfman, NAMRI; Brian Greenwood, British MRC, The Gambia, West Africa; Richard Haughton, Scripps Clinic, LaJolla, CA; Alan Cochrane, NYU Medical Center, NY, NY.

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	0.6	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Circumsporozoite (CS) proteins have been shown to act as the protective antigens capable of inducing sporozoite - specific immunity in several species of malaria. The gene coding for this antigen in *Plasmodium falciparum* has been isolated, cloned, and incorporated into *E. coli*. The recombinant protein induces antibodies in mice which react with sporozoites and blocks liver invasion *in vitro*.

T cells are important for the development of protective immunity to malarial sporozoites. The repeated epitope, NANP, of the *P. falciparum* circumsporozoite (CS) protein is recognized by T cells from mice which carry the I-A^b gene. Evidence suggests that the human immune response to this epitope may be very restricted. This has serious implications for vaccine development.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00161-10 LPD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunochemistry of Parasitic Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.E. Nash	Medical Officer	LPD, NIAID
Others:	F.A. Neva		
	R.A. Adam	Medical Staff Fellow	LPD, NIAID
	A. Aggarwal	Visiting Fellow	LPD, NIAID

COOPERATING UNITS *(if any)*

M.M. Levine, D.A. Herrington, G.A. Lysonsky, University of Maryland

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.3	1.8	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Antigenic variation of Giardia lamblia was shown by studying organisms derived from cloned populations. Cloned Giardia (WB) were exposed to cytotoxic antibodies and the surviving organisms analyzed. Surviving organisms (progeny) differed from their parent and many differed amongst themselves as judged by IFA, Western blots, cytotoxicity assays, surface labeling, and reactivity of excretory-secretion products with specific antisera. In order to detect further changes, another cytotoxic MAb (5C1) was reacted with one of the progeny and resistant organisms studied. Antigens reactive with 5C1 were not present in the original WB parent or the 5C1 resistant progeny. 170 kd antigen was only found in the original WB parent. Loss of 170 kd antigen was very rapid, about $1/10^3$. DNA sequence data showed the 170 kd antigen was cysteine rich. This was confirmed by in vitro labeling with labeled cysteine. Furthermore, one of the antigens which replaced the 170 kd antigen was also labeled with cysteine. It may be that this type of protein composition is required for surface antigens which vary in Giardia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00162-11 LPD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Cytology of Host-Parasite Interactions in Parasitic Protozoa

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D.M. Dwyer	Supervisory Microbiologist	LPD, NIAID
Others:	P.A. Bates	Visiting Fellow, FIC	LPD, NIAID
	I. Bonsch	Guest Investigator	LPD, NIAID
	M.O. Odera	Guest Investigator	LPD, NIAID
	K.B. Pastakia	Senior Staff Fellow	LPD, NIAID

COOPERATING UNITS (if any) Dept. Immunol. & Infect. Dis., Johns Hopkins Univ., (M. Gottlieb); Dept. Biochem, Univ. Victoria (R.W. Olafson); Dept. Biol., Technion-Isr. Inst. Tech. (D. Zilberstein); Inst. Med. Parasit., Univ. Bonn (I. Bonsch)

LAB/BRANCH

Laboratory of Parastiic Diseases

SECTION

Cell Biology & Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.8	4.6	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The cell biology and biochemistry of Leishmania and Trypanosoma are investigated as models of intra- and extracellular parasitism, respectively. As all interactions between host and parasite occur at the level of the parasite surface membrane (SM), emphasis is placed on: 1) its integrated biochemical and physiological characterization and 2) defining its roles in parasite survival and development.

A 76 KDa SM glycoprotein, with putative carboxyl-carrier function was identified in L. donovani (Ld) promastigotes. A SM L-proline-binding/transport protein containing two subunits of 41 and 34 KDa was identified in Ld. The biosynthetic secretory pathway of soluble-acid phosphatase (S-AcP) was localized in both Ld pro- and amstigotes. S-AcP was purified and the first 30 residues of its N-terminal amino acid sequence determined. Oligonucleotide probes based on this sequence were used to identify and clone the S-AcP gene. The inducible Ld SM 3'-nucleotidase/exo-ribonuclease was isolated, purified and shown to be a 43 kDa mannosylated glycoprotein. Antibodies are being used to screen cDNA-expression libraries to identify and clone its gene. The LD SM 5'-nucleotidase was isolated, purified and shown to be a mannosylated glycoprotein containing two subunits of 72 and 43 kDa. Further, the LD SM glucose transport protein and proton-ATPase were identified and characterized. Based on their homologies with similar proteins in other systems, oligonucleotides of conserved functional regions were synthesized as probes for identifying and cloning the genes for these two leishmanial proteins.

The current results provide further functional characterization of parasite SM constituents and these could serve as potential targets for chemotherapy and/or immunodiagnosis/-prophylaxis.

PHS 6040 (Rev. 1/84) GPO 914-918

LMSF

LPB

LPVD

RMOB

LVD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00197-08 LPD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunoregulation and Immune Recognition in Filariasis and Non-filarial Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.B. Nutman	Medical Officer	LPD, NIAID
Others:	E.A. Ottesen	Senior Investigator	LPD, NIAID
	R.B. Lal	Visiting Scientist	LPD, NIAID
	R.T. Davey, Jr.	Medical Staff Fellow	LCI, NIAID
	D.J. Ward	Medical Staff Fellow	LPD, NIAID
	D. Freedman	Medical Staff Fellow	LPD, NIAID
	R.G. Crystal	Chief	PB, NHLBI

COOPERATING UNITS *(if any)* Indian Council of Medical Research Centre, Madras, India (V.Kumaraswami, P.R. Narayanan, R. Paranjape); Dept. Allergy and Immunology, SUNY-Stony Brook, Stony Brook, NY (D.J. Volkman); Dept. Clinical Immunology, U. Montreal, Cooperating Units Continued on last page.

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Clinical Parasitology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	0.6	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to delineate the mechanisms involved in regulating the humoral and cellular responses in patients with filariasis and other disease states and to determine those antigens responsible for inducing these responses.

Immunoregulatory studies have examined the phenomenon of antigen-specific anergy in microfilaremic patients by demonstrating this anergy at the level of antibody (B cell) and lymphokine (T cell) responsiveness. Further, similar T cell anergy has been found among patients with active onchocerciasis. The influence of genetic factors and antigen composition on this anergy has begun using molecular biologi-cal tools and defined antigens.

In vitro models of parasite-antigen driven antibody production as well as parasite-specific and HTLV-I transformed T cell clones have been developed to understand in more detail those mechanisms regulating antibody production (particularly IgG and IgE) in filarial and non-filarial diseases.

Qualitative analysis of filaria-specific IgE and IgG in loiasis, lymphatic filariasis, and onchocerciasis have indicated patterns of antigen recognition which differ among groups of patients with different clinical manifestations of filariasis. Using these techniques, antigens recognized exclusively by patients with loiasis or onchocerciasis have been identified. Filarial species-specific antigens recognized by human monoclonal antibodies derived from EBV-transformed patient B cells have also been identified and characterized. Further, FPLC fractionation and qualitative analysis of the antigenic components of filarial parasites has identified two sets of proteins which appear to regulate the immune response at both the cellular and humoral level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00208-07 LPD

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Isolation and Characterization of Plasmodial Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.F. McCutchan	Microbiologist	LPD, NIAID
Others:	A. Lal	Visit. Assoc.	LPD, NIAID
	V. de la Cruz	Staff Fellow	LPD, NIAID
	K. Vernick	Technician	LPD, NIAID
	A. Waters	Visit. Assoc.	LPD, NIAID
	J. Welsh	Technician	LPD, NIAID
	W. Weiss	Guest Researcher	LPD, NIAID
	M. Good	Visiting Scientist	LPD, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 4.0	PROFESSIONAL: 3.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This laboratory has been involved in contributing to the development of a malarial vaccine. To this end we have been characterizing surface antigen genes from Plasmodium falciparum and have been developing a rodent model for vaccine testing. An antigen corresponding to the sporozoite antigens of the human malarias has been identified in a rodent malaria and a rodent model for malaria vaccines has been developed. We have immunized mice against this antigen. We have challenged the immunized mice with malarial parasites and characterized the degree of protection afforded by the vaccination as well as the B-cell and T-cell responses.

We have also identified and characterized a major biochemical step in the development of Plasmodium , the stage-dependent ribosome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00240-06 LPD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Antigenic Analysis of Sexual Stages of Malaria Parasites

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L.H. Miller	Head, Malaria Section	LPD, NIAID
	T.F. McCutchan	Microbiologist	LPD, NIAID
Others:	D. Kaslow	Guest Worker	LPD, NIAID
	C. Syin	Guest Worker	LPD, NIAID
	I. A. Quakyi	Guest Worker	LPD, NIAID
	D. Keisnter	Biologist	LPD, NIAID
	J. Coligan	Branch Chief	BRB, NIAID

COOPERATING UNITS *(if any)* U. of Colombo, Sri Lanka (K. Mendis); Harvard Sch. of Pub. Hlt., Boston (D. Wirth); Papua New Guinea Inst. Med. Res. (P. Graves); Naval Med. Res. Inst., Bethesda, MD (A. Szarfman); Hazelton Laboratoires, VA (J. Rener); U. of Edinburgh, Scotland (R. Carter); Sch. of Pub. Hlth, Hopkins U. (N. Kumar)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Malaria Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.7	3.2	1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Identify gamete and ookinete proteins and clone the genes encoding these protein antigens which are the targets of antibodies blocking infectivity of gametocytes to mosquitoes. Protein targets of immunity will be identified with monoclonal antibodies. The cloning will depend on purification of target antigens with monoclonal antibodies and the microsequencing of peptic fragments from these proteins in order to make oligonucleotide probes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00241-06 LPD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Identification of Receptors for Merozoite Invasion of Erythrocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L.H. Miller	Section Head	LPD, NIAID
Others:	F. Klotz	Staff Fellow	LPD, NIAID
	D. Hudson	Biologist	LPD, NIAID
	J. Dalton	Visiting Fellow	LPD, NIAID
	R. Howard	Expert Consultant	LPD, NIAID

COOPERATING UNITS (if any) WRAIR, Washington, DC (J.D. Haynes); Hazelton Laboratories, Vienna, VA (J. Rener); Case Western Reserve Univ., Cleveland, OH (M. Aikawa); Univ. of Kentucky Sch. of Med. (T. Hadley)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Malaria Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The merozoite interacts in a receptor specific manner with the erythrocyte surface and is the stage against which immunity may work to block invasion. Thus, merozoite surface components are of interest for their role in erythrocyte recognition and as antigens for induction of protective immunity. We are identifying P. knowlesi receptors for attachment to monkey and human erythrocytes. We are studying one antigen on the merozoite surface that undergoes antigenic variation to understand the molecular basis for this variation.

We are studying P. falciparum mutants that use an alternative receptor for invasions of human red cells. We are attempting to adapt P. falciparum to subprimates as an animal model for vaccine trials.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00242-06 LPD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biological and Biochemical Studies of Antigens on Malaria-Infected Red Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Howard	Visiting Scientist	LPD, NIAID
Others:	L.H. Miller	Section Head,	LPD, NIAID
	L.J. Panton	Guest Worker	LPD, NIAID
	A.S. DeGroot	Clinical Associate	LPD, NIAID
	W. Daniel	Research Associate	LPD, NIAID

COOPERATING UNITS *(if any)* Case Western Reserve Univ. (M. Aikawa, C. Atkinson); LC NIAMD (K. Kirk, L. Cohen); Georgetown Univ. (D. Taylor, M. Small); Christian-Albrecht's Univ., W. Germany (R. Schamer, G. Reuter); U. of Cal., San Fran. (J. Leach); DNAX, Palo Alto (T. Yokota, K. Arai).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are studying the structure and function of malarial proteins inserted into and through the membrane of erythrocytes infected with mature asexual malaria parasites and molecules on the endothelial cells involved in cytoadherence.

Five malarial proteins have been localized to the membrane of Plasmodium falciparum-infected erythrocytes at the mature trophozoite stage. The M_r ~300,000 cell surface protein associated with the property of cytoadherence to endothelial cells (denoted Pf EMP 1, P. falciparum erythrocyte membrane protein 1) has been shown to be antigenically distinct from another very large (M_r ~300,000) malarial protein located under the surface membrane in the electron-dense material at knobs (denoted Pf EMP 2). The histidine-rich protein (HRP) associated with knob expression (denoted PfHRP 1) was also shown to be localized under the membrane in the electron-dense material. Another HRP that is exported from infected cells into plasma (called Pf HRP 2) is also associated with the surface membrane, presumably en route for secretion since it is accessible to antibody at the cell surface and also attached to the cytoskeleton. We also showed that RESA, a protein inserted into the host membrane during merozoite invasion, remains in the membrane and is accessible to lactoperoxidase catalyzed radioiodination with trophozoite-infected cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00244-06-LPD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Developmental Adaptation of Trypanosoma cruzi to the vertebrate immune system.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Sher	Section Head	LPD, NIAID
Others:	K. Joiner	Senior Investigator	LCI, NIAID
	R. Da Silva	Fogarty Fellow	LPD, NIAID
	S. Heath	Fogarty Fellow	LPD, NIAID
	M. Grimaldi	Fogarty Fellow	LCI, NIAID
	D. Sacks	Senior Investigator	LPD, NIAID

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Immunology and Cell Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 2.5	PROFESSIONAL: 2.0	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In this project we have been studying developmental adaptations of T. cruzi to the vertebrate host, and in particular, the molecular basis of adaptive changes occurring during the differentiation of epimastigotes (vector stage) to metacyclic trypomastigotes (infective stage).

A. Characterization of a stage-specific factor which accelerates complement decay
Culture supernatants from metacyclic and tissue culture trypomastigotes were shown to contain an activity which both accelerates the decay of and inhibits the formation of complement C3 convertases. This factor was characterized for its sensitivity to enzymes, its binding to lectins, and was shown to be contained within a group of molecules with M_r's ranging form 80-90 K.

B. Analysis of stage-specific gene expression in metacyclic trypomastigotes
In an effort to identify genes whose expression is turned on during the differentiation of infective stage parasites, mRNA was extracted from metacyclic trypomastigotes and epimastigotes and translated in vitro. Analysis by immunoprecipitation with stage-specific antisera revealed several gene products uniquely expressed in metacylic trypomastigotes. A cDNA library was constructed from the metacyclic mRNA to clone examples of these stage-specific gene products.

C. Studies on developmental control of intracellular survival in macrophages
A model was established in which metacyclics developing in culture were shown to survive in mouse peritoneal macrophages while log phase epimastigotes are killed. Pre-treatment with mouse complement was shown to enhance parasite survival presumably by channelling uptake into "non-toxic" intracellular compartments.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00246-05 LPD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Studies of the Genome and Surface of Schistosoma mansoni

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Sher	Section Head	LPD, NIAID
Others:	E. J. Pearce	Guest Worker	LPD, NIAID
	D. Lanar	Guest Worker	LPD, NIAID
	P. Brindley	Fogarty Fellow	LPD, NIAID
	L. Hall	Medical Staff Fellow	LCI, NIAID

COOPERATING UNITS *(if any)*

Biomedical Research Institute, Rockville, MD (F. Lewis); University of Iowa (J. Donelson, K. Brown); Vanderbilt University (R. Zimmerman)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of this project is characterize schistosome surface molecules relevant to immunity, to analyze the genome of the parasite in relation to specific biological properties of the organism and to clone important schistosome immunogens.

A. Molecular biology of paromyosin. In order to complete the nucleotide sequence of the paramyosin immunogen from S. mansoni, several genomic clones were isolated. In related studies, paramyosin from S. japonicum was cloned from a λgt-11 expression library.

B. Molecular analysis of hycanthone resistance. Further restriction fragment length polymorphisms (RFLP) were identified in the DNA of schistosomes with experimentally induced resistance to hycanthione using different restriction enzymes. The RFLP's appeared to correlate with resistance since they re-occurred when a new resistant line was derived from the drug sensitive parent. In addition, differences were noted in the in vitro translation products of mRNA from drug sensitive and resistant lines.

C. Autologous control of complement activation by the schistosome membrane. The aim of this new project is to understand how schistosomes are able to control activation of the alternative complement pathway (ACP) of the vertebrate host. It was shown that this control is autologous, i.e., worms recovered from one host fail to activate complement of that species but do activate complement of unrelated species. Moreover, treatment with either neuraminidose or phospholipase C was shown to convert schistosomes into activators of autologous complement. The data suggests that the molecules required for control of autologous complement activation are acquired by the schistosome membrane from the host.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00248-06 LPD

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetics & Physiology of Vector Capacity in Anopheline Mosquitoes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R.W. Gwadz	Senior Scientist	LPD, NIAID
	L.H. Miller	Section Head	
	R.K. Sakai	Expert	
Others:	P.A.J. Romans	Visiting Fellow	
	K.D. Vernick	Bio. Lab. Tech	
	D. Kaslow	Med. Staff Fellow	
	J. Lee	Visiting Fellow	

Others continued on last page

COOPERATING UNITS (if any)

Dept. of Entomology, U. of Maryland, Colleage Park, MD (Dr. M. Ma); Dept. of Animal Genetics, U. Edinburgh, (Dr. R. Carter)

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Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.9	2.9	4.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The molecular biology of anopheline mosquitoes is being studied in relation to the capacity of these vectors to transmit malaria. Systems for cloning and transposing genes into mosquito germ lines are being developed. Factors which render mosquitoes refractory to malarial infection, with special emphasis on mechanisms which encapsulate developing parasites, are being defined. Linkage of refractory genes to genes regulating blood-meal associated physiological events, e.g., vitellogenin synthesis, will be used to enhance refractory responses.

The ability to identify, clone, and transpose genes which regulate refractory mechanisms should permit the development of mosquito lines incapable of transmitting malaria for use in malaria control schemes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00251-06 LPD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunologic Studies on Schistosomiasis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Sher	Section Head	LPD, NIAID
Others:	E.J. Pearce	Guest Worker	LPD, NIAID
	P. Brindley	Fogarty Fellow	LPD, NIAID

COOPERATING UNITS *(if any)*
George Washington University (S.L. James); Johns Hopkins University (M. Strand)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Immunology and Cell Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	2.0	0.8

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of this project is to study mechanisms of immunity and immune evasion in schistosomiasis with the ultimate goal of developing an experimental vaccine employing defined antigens.

A. Vaccination trials with purified paramyosin. Affinity purified or biochemically isolated paramyosin was shown to confer significant protection against challenge infection in 10 out of 12 experiments employing intradermal vaccination with BCG while vaccination with schistosome myosin or heterologous paramyosin was ineffective. However, schistosome extracts depleted of paramyosin were also protective suggesting the existence of other important immunogens in the intradermal model.

B. Identification of E/S antigens recognized by vaccine antibodies. Metabolically labeled lung-stage schistosomula were shown to release four antigens of 30, 36, 64, and 100 Kd which are specifically recognized by mice multiply vaccinated with irradiated cercariae. A monoclonal antibody was produced against the 100 Kd component. These antigens are being investigated as new candidate vaccine immunogens.

C. Dependence on antibody of praziquantel therapy. By assaying the binding of monoclonal antibodies to praziquantel treated worms recovered from athymic mice, an antigen of 200 Kd was identified which is specifically exposed as a result of drug treatment and therefore is a candidate target for the antibody response previously shown to be required in praziquantel therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00253-06 LPD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of the Immunologic Responses to Filarial Helminth Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.A. Ottesen	Senior Investigator	LPD, NIAID
Others:	R. Hussain	Visiting Fellow	LPD, NIAID
	T.B. Nutman	Medical Officer	LPD, NIAID
	D. Ward	Medical Staff Fellow	LPD, NIAID
	R. Davey	Medical Staff Fellow	LCI, NIAID
	D. Freedman	Medical Staff Fellow	LPD, NIAID
	R. Lal	Visiting Fellow	LPD, NIAID
	T.M. Chused	Senior Investigator	LI, NIAID

COOPERATING UNITS *(if any)* Harvard Med.Sch. (S.Ackerman); Walter Reed Army Inst.Res. (M. Grögl); PB, NHLBI, NIH (R.G.Crystal & W.Rom); CB, NEI, NIH (R.Nussenblatt & C.C.Chan); Indian Council of Med.Res. Tuberculosis Res. Centre, Madras, India (S. P.Tripathy, P.R. Narayanan, V. Kumaraswami, R. Paranjape, V. Vijayan;

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host Parasite Relations

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.05	4.05	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to define the humoral and cellular immune responses that relate to immunopathology, protective immunity and immunodiagnosis of patients with lymphatic filariasis and onchocerciasis.

Antibodies of the IgG subclasses appear differentially associated with the clinical manifestations of bancroftian filariasis; patients with elephantiasis have prominent IgG_3 antifilarial antibodies and minimal IgG_4 responses while the opposite is true for those with asymptomatic infection.

Studies of eye tissue and fluid from patients with ocular onchocerciasis show active local IgG antibody production but little or no indication of a pathogenetic role for IgE in these lesions. T-cell infiltration of the iris is of the suppressor/cytotoxic phenotype (Leu 2a), opposite to the findings in most uveitis syndromes but consistent with the general parasite-specific, primary T-cell immunosuppression demonstrated in both onchocerciasis and lymphatic filariasis.

The eosinophil granule protein MBP, important in the pathology of helminth infections, can now be defined in tissues by immunofluorescence and in fluids by immunoassay with monoclonal antibodies.

Populations with onchocerciasis or bancroftian filariasis have been examined to define immunologic parameters that distinguish 'naturally' immune from infected individuals. A 43kD protein from infective larvae appears as a potential protective immunogen for bancroftian filariasis, and specific probes are being generated to study it further.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00255-06 LPD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of the Immunologic Responses to Non-Filarial Helminth Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.A. Ottesen	Senior Investigator	LPD, NIAID
Others:	R. Davey	Medical Staff Fellow	LPD, NIAID
	D.O. Freedman	Mecial Staff Fellow	LPD, NIAID
	T.B. Nutman	Medical Officer	LPD, NIAID
	J. White	Special Assistant	OSD, NIAID

COOPERATING UNITS *(if any)*

Howard University (C. Maxwell)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host Parasite Relations

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Experimental hookworm (Necator americanus) infections in normal volunteers have now been terminated. It was found that a small infecting dose of 50 filariform larvae was capable of producing signficant transient gastrointestinal morbidity and marked blood eosinophilia but induced other T-cell and B-cell dependent immune responses that were of such low magnitude as to be difficult to evaluate extensively. Clear changes in eosinophil structural and functional profiles could be documented during the course of the infections. With a newly developed assay for the eosinophil granule protein MBP, analysis of serum and tissue samples collected during infection continues in order to establish the mechanistic implications of the eosinophil response to helminth infections.

To account for the neo-arteriolization of the peri-portal fibrotic tissue around the eggs of Schistosoma mansoni in the livers of infected humans, parasite products were evaluated for endothelial cell growth factor activity. Very potent endothelial cell activation and proliferation were induced by soluble antigens from eggs but not adult schistosomes, all in the absence of any immune cells or factors.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00256-06 LPD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Differentation of Leishmania Promastigotes

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. Sacks	Senior Investigator	LPD, NIAID
Other:	R. daSilva	Fogarty Fellow	LPD, NIAID
	S. Puentes	Clinical Staff Fellow	LCI, NIAID
	L. Hall	Clinical Staff Fellow	LCI, NIAID
	K. Joiner	Senior Investigator	LCI, NIAD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects x ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The differentiation of Leishmania promastigotes from a non-infective to an infective or metacyclic stage is accompanied by changes in surface carbohydrates as detected by the lectin PNA. This change is a consequence of the sialation of a cell surface and released glycolipid, which can be identified by the monoclonal antibody 3F12, which is specific for metacyclic promastigotes of L. major. The expression of the modified form of the glycolipid appears to influence the type of receptors on the macrophage which the organisms use for attachment and uptake. Metacyclic promastigotes can be shown to use CR1 receptors, because their attachment can be inhibited by mAb against these receptors. Non-infective promastigotes from log phase cultures cannot be shown to utilize C3 receptors. Opsonization of promastigotes with C3 enhances their uptake and promotes their survival, indicating that the choice of receptors for attachment influences subsequent intracellular survival.

The molecular nature of these receptor ligand interactions and consequences will be the basis of further studies in terms of the role of the glycolipid and in particular the sialic acid, the role of other cellular receptors for carbohydrates, and the role of complement activation and C3b deposition in these events.

LMSF LPB LPVD RMOB LVD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00257-06 LPD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)
Immunology of Strongyloidiasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	F.A. Neva	Chief	LPD, NIAID
Others:	P.J. Brindley	Visiting Fellow	LPD, NIAID
	E. Pearce	Guest Research	LPD, NIAID
	K. Barrett	Visiting Fellow	LCI, NIAID
	W.T. London	Chief, Expt. Path. Sect.	IRP, NICDS
	T. Nash	Senior Investigator	LPD, NIAID
	E.A. Ottesen	Head, Clinical Parasitol. Sect.	LPD, NIAID

COOPERATING UNITS (if any)

SEMA, Inc., Rockville, MD (G. Phillips); Merck, Sharpe, Dohme; Rahway, N.J. (K. Brown); Univ. of California at San Francisco, Dept. of Pathology. (J. McKerrow).

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Cell Biology and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	0.7	0.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project involves clinical and immunologic studies of humans infected with the intestinal nematode Strongyloides stercoralis, and parallel studies in an experimental host, the patas monkey. This parasite deserves special investigative attention because it often goes undiagnosed, it can be fatal in immunosuppressed individuals, and has unusual biologic properties.

During maintenance of the parasite in the monkey during last year we evaluated and found once again that the immediate hypersensitivity skin test elicited nonspecific reactions in the monkey and was not useable. One infected animal was repeatedly exposed to infective larvae to obtain a hyperimmune serum. Most effort on the project was given to analysis of somatic and excretory-secretory (E/S) antigens prepared from infective (L3) larvae. Only antigens that react with IgG from pooled patients' sera can be identified by immunoblotting. Repeated attempts to detect antigens by Western blot, as well as dot blot, that react with IgE have been negative. Yet we believe that allergic reactions to strongyloides antigens (as assayed by in-vitro histamine release) are mediated by IgE. Support for this comes from an experiment in which heating serum of patients before in-vitro passive sensitization of donor basophils ablated ability of antigen to release histamine. Additional probes to identify and characterize the various larval antigens are being developed--some fractions show protease activity and polyvalent rabbit antisera that react with parasite antigens have been raised. Several patients with strongyliodiasis have been encountered who have antibody evidence of infection with the retrovirus HTLV-1.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00347-05 LPD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on the Shistosomal Hepatic Fibrosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. W. Cheever	Assistant Chief	LPD, NIAID
Others:	S. Deb	Bio. Lab. Tech	LPD, NIAID
	R. H. Duvall	Bio. Lab. Tech. (Micro.)	LPD, NIAID
	J. Malley	Mathematical Statistician	LSM, DCRT
	A. Sher	Head, Immunology Section	LPD, NIAID
	R. Minker		LSM, DCRT

COOPERATING UNITS *(if any)*

Brigham, Women's Hospital, Boston, MA, Dept. of Pathology (F. V. Lictenberg, J. Bryam)

LAB/BRANCH

Laboratory of Laboratroy of Parasitic Diseases

SECTION

Host-Parsite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.50	0.70	0.80

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hepatic fibrosis is examined in mice infected wtih schistosome species pathogenic for man. Mouse strains developed markedly different degrees of hepatic fibrosis following infection with S. mansoni. T cells are important for the formation of granulomas in both S. mansoni and S. japonicum infected mice, as determined from examination of athymic and B cell depleted mice. Current studies are focused on determining which subsets of T cells are involved in regulation of S. japonicum egg granulomas. BALB/c mice are not much more responsive to S. japonicum eggs than are BALB/c nu/nu mice, precluding studies of cell transfer in this strain, BALB/c mice do have more fibrosis than do their nude counterparts, emphasizing the role of T cells in hepatic fibrosis in this system and providing one more example of the dissociation of granulom size and fibrosis. T cells are required for the formation of normal granulomas, as indicated by marked differences between NCR outbred nu/nu versus nu/+ and between C57Bl/6 nu/nu versus C57Bl/6. Normal L3T4 cells or Ly2.2 cells from mouse spleen were transferred to C57BL/6 nude mice. Both cell types restored the formation of granulomas of normal size in mice subsequently infected with S. japonicum.

LMSF
LPB
LPVD
RMOB
LVD

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00348-04 LPD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on the Immunity in Murine Shistosomias

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. W. Cheever	Assistant Chief	LPD, NIAID
Others:	R. H. Duvall	Bio. Lab. Tech. (Micro.)	LPD, NIAID
	A. Sher	Section Head	LPD, NIAID
	J. Malley	Mathematical Satistician	LSM, DCRT
	K. Malley	Computer Assistant Analyst	LSM, DCRT
	P. Shade	Biol. Lab. Tech.	LPD, NIAID

COOPERATING UNITS *(if any)*

Biomedical Research Institute, Rockville, MD (F. Lewis and C. Richards)
George Washington University (S. James)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Host-Parasite Relations Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00350-05 LPD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
DNA Analysis of Parasites

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.E. Nash	Medical Officer	LPD, NIAID
Others:	A. Aggarwal	Visiting Fellow	LPD, NIAID
	R. Adam	Medical Staff Fellow	LPD, NIAID
	A. Lal	Guest Researcher	LPD, NIAID
	T. McCutchen	Senior Scientist	LPD, NIAID
	P. Romans	Staff Fellow	LPD, NIAID
	T. Wellems	Staff Fellow	LPD, NIAID
	V. de la Cruz		

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Host-Parasite Relations Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.7	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Giardia undergo antigenic variation. Cloned populations contain small numbers of Giardia with unique surface antigens and these can be selected for by exposing Giardia to cytotoxic MAbs which react with original major antigen. Antigens present in the original isolate are not present in subsequent progeny and later antigens are not present in the original isolate. The major antigen of the original WB isolate (170 Kd) and one of the newly detected surface antigen are cysteine containing compounds. The DNA of clones of the original isolate (parents) were compared to the DNA of populations which grew following exposure to cytotoxic MAb (progeny). Multiple bands were seen in Southern blots using part of the cloned 170 kd antigen as a probe. Most progeny differed from each other but parent-progeny pairs were the same. Although frequent rearrangements were seen in the area encoding for this varying antigen, the differences in the banding patterns did not correlate with the loss of the major antigen. The major antigen of the parent isolate is a 170 kd antigen. Part of this gene was sequenced and showed two tandem repeats (65 amino acids) and part of a third. The entire segment has 12% cysteine.

A Giardia gene was identified which hybridized under non-stringent conditions to hsp-70 of Drosphila. Sequence analysis of a 2 kb fragment revealed an open reading frame but no similarity to hsp-70. The promotor region had the typical heat shock promotor.

The chromosomes of Giardia were identified using pulse field electrophoresis. Five chromosomes were identified and a number of minor less brightly staining Giardia DNA containing bands. Isolates differ in the position of the major bands and the number and position of the minor bands. Clones of the same isolate differ in the position of the minor bands.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00351-05 LPD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Parasite and Host Factors Controlling the Pathogenesis of Leishmaniasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P.A. Scott	Senior Staff Fellow	LPD, NIAID
Other:	A. Sher	Section Head	LPD, NIAID
	F.A. Neva	Chief	LPD, NIAID
	P. Natovitz	Biologist	LPD, NIAID
	D. Dwyer	Supvr. Microbiologist	LPD, NIAID
	E. Pearce	Guest Researcher	LPD, NIAID
	S. Heath	Guest Researcher	LPD, NIAID

COOPERATING UNITS (if any)

George Washington University, Washington, DC (S. James)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A vaccine against Leishmania major in BALB/c mice using a soluble fraction of the parasite was developed. This soluble extract was further fractionated by an FPLC Mono Q column into subfractions with differing capacities to stimulate T cells and to be recognized by antibodies. Only proteins within one subfraction (fraction 9) were capable of inducing protection, although other fractions were recognized by T lymphocytes from immunized mice. While the qualitative differences between T cell antigens which do and do not induced protection are unknown, it is possible that distinct macrophage subsets may be elicited by different T cell populations. For example, we found that macrophages from non-immunized infected BALB/c mice are relatively unresponsive to activation signals, while macrophages from immunized mice are capable of killing the intracellular parasite. Current studies are focussed on identification of the protective immunogen(s) within fraction 9. Since immunity in this model has been shown to be mediated by T cells, identification of the fraction 9 molecules recognized by T cells may serve this purpose. Consequently, T cell lines and clones reactive against antigens within fraction 9 were produced, and preliminary results indicate that the T cell lines can passively protect naive mice. The antigen specificity of these T cells was determined by separation of leishmanial antigens by SDS-PAGE, immunoblotting to nitrocellulose, and stimulation of T cells with small sections of the blot corresponding to defined molecular weights. Antigens in the relative molecular weight regions of 8 K to 14 K, 20 K to 30 K and 40 K to 45 K were found to stimulate the T cell line. When T cell clones were tested, one clone was found that responded specifically to the 8 K and 10 K proteins. These molecules are currently being eluted from an SDS polyacrylamide gel, and are being tested for their immunoprophylactic capacity. Finally, a polyclonal rabbit antisera raised against fraction 9 is being used to screen a Leishmania λgt11 library for recombinants expressing proteins found within this protective fraction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00439-03 LPD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Clinical and Therapeutic Studies in Human Filariasis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.A. Ottesen	Senior Investigator	LPD, NIAID
Others:	T.B. Nutman	Medical Officer	LPD, NIAID
	D.O. Freedman	Medical Staff Fellow	LPD, NIAID
	T.E. Nash	Senior Investigator	LPD, NIAID
	R. Davey	Medical Staff Fellow	LCI, NIAID

COOPERATING UNITS *(if any)*
PB, NHLBI, NIH (R.G. Crystal); Tuberculosis Research Centre, Madras, India; (R. Prabhakar and V. Kumaraswami); Madras Medical College, Madras, India (V. Vijayasekaran); Peace Corps Medical Office (M. Mulligan).

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Host Parasite Relations

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Ivermectin has been shown in an open, in-hospital trial in South India to be effective in clearing microfilaremia in patients with bancroftian filariasis at dosages as low as 25-mcg/kg given once orally. A second, blinded, placebo-controlled trial of invermectin and the currently used drug, diethylcarbamazine (DEC), has completed its treatment but not follow-up phase. Preliminary findings suggest that the efficacy of Ivermectin and DEC are equivalent and that the side reactions induced by ivermectin may be significantly less. These findings and invermectin's single-oral-dose mode of administration make it an excellent candidate to replace DEC as the drug of choice for treating lymphatic filariasis.

Because long-term follow-up studies employing bronchoalveolar lavage to evaluate patients with TPE treated conventionally with diethylcarbamazine (DEC) have shown that ~ 1/3 relapse or have persistent low-grade alveolitis leading to pulmonary fibrosis, a therapeutic trial of three regimens (conventional DEC, long-term DEC, or DEC $^+$ steroids) has been initiated in Madras. The study will require about three yeas to enroll the anticipated 75 study patients.

Observations on 200 Peace Corps volunteers going to Loa loa endemic areas of Africa are concluding after three years of a placebo-controlled chemoprophylaxis trial using weekly doses of DEC. Seroconversion is at ~ 15% but final analysis of the results is not yet complete.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00483-02 LPD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetics of Malaria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Thomas E. Wellems	Staff Fellow	LPD, NIAID
Others:	Lindsey J. Panton	Fogerty Fellow	LPD, NIAID
	Photini Sinnis	Visiting Fellow	HHMI
	Robert Gwadz		LPD, NIAID

COOPERATING UNITS *(if any)* Biomedical Research Institute (Virgilio E. do Rosario); Dept. Experimental Therapeutics, WRAIR (Ayo M.J. Oduola, Wilbur Milhous); Dept. Genetics, University of Edinburgh (Brian Fenton, David Walliker); Naval Institute of Medical Research (Ana Szarfman).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Genetic crosses of cloned parasite lines, restriction fragment length polymorphism (RFLP) analysis, and chromosome mapping techniques are being used to investigate linkage groups and genes that affect drug susceptibility and red cell invasion in Plasmodium falciparum. Chromosome markers exhibit such diversity between different parasite strains that linkage groups can be readily analyzed for association with parental pheontypes in genetic crosses. Using this approach, we have found that one end of chromosome 13 contains genes which affect erythrocyte-stage proliferation in vitro. Investigations will now be directed at determining exactly which factors expressed by this region of the genome account for differences in proliferation.

A similar approach is being used to analyze the problem of drug resistance in malaria. A genetic cross between a multi-drug resistant and a drug sensitive clone of P. falciparum is being carried out. Techniques of reverse genetics will be applied to progeny of the cross in an effort to identify and characterize linkage groups that correlate with drug resistance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00487-01 LPD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on the Quantitative Parasitology of Schistome Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. W. Cheever	Assistant Chief	LPD, NIAID
Others:	R. H. Duvall	Bio. Lab. Tech. (Micro.)	LPD, NIAID
	S. Deb	Bio. Lab. Tech.	LPD, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.3	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Maturation of Schistosoma japonicum in mice was found to be highly variable. Many worms had matured and begun to lay eggs 4 weeks after infection, but other worms did not mature until the 6th week after infection and many worms never matured. 500 rad whole body irradiation, given before infection, slowed the maturation of S. japonicum but not that of S. mansoni. This effect is likely to have an immune basis, as it is largely reversed by passive transfer of spleen cells after irradiation. Other findings relate to the demonstration, by others, of an inhibitory effect of extra male or female S. mansoni on egg production of worm pairs in mice and baboons. We were unable to detect such an effect in mice or in retrospective analysis of extensive data from rhesus monkeys or infected humans. Analysis of previous data from capuchin monkeys infected with S. haematobium and from rabbits infected wtih S. japonicum also failed to show such an effect. Ongoing studies deal with the destruction of S. japonicum eggs in the tissues of mice and with the toxic effects of praziquantel on schistosome eggs in the tissues of mice. Excretion of eggs in the feces is being examined in relation ot the immune status of the mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 00494-01 LPD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of T Cell Responses in Human Leishmaniasis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. Sacks	Senior Investigator	LPD, NIAID
Others:	P. Melby	Clinical Staff Fellow	LPD, NIAID
	Frank Neva	Chief	LPD, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Control and resolution of leishmanial infection depends primarily on T cell mediated immune mechanisms. The nature of the leishmanial antigens involved in eliciting T cell immunity is unknown. We have examined the pattern of peripheral blood lymphocyte responses in patients with active, healed, or subclinical leishmanial infection to fractionated leishmanial antigens using a T cell immunoblotting method. Leishmanial soluble antigens are separated by SDS-PAGE and electroblotted to nitrocellulose which is then punched into individual wells of a 96-well tissue culture plate. Lymphocytes are then cultured with the nitrocellulose-bound antigen and proliferative responses measured.

Cells from patients with mucosal and cutaneous leishmaniasis, and healed cutaneous *L. donovani* infection respond in a profoundly hetergeneous pattern to antigens of molecular weights ranging from 5-200 kD. Peak proliferative responses occurred to very low molecular weight antigens (10-20 KD). In addition, significant lymphoctye proliferative repsonses from subjects with past subclinical *L. donovani* infection were found to be restricted to a limited number of low molecular weight antigens.

The method has also been used to identify the antigens which are recognized by T cell clones which have been generated from the peripheral cells of a patient with mucocutaneous leishmaniasis. We hope to apply the method to the identification of antigens recognized by T cell clones form individuals re active to *L. donovani* as a consequence of asymptomatic or subclinical infection.

LMSF

LPB

LPVD

RMOB

LVD

LABORATORY OF VIRAL DISEASES
1987 ANNUAL REPORT
Table of Contents

SUMMARY 15-1

Project Number

Z01 AI 00020-12 LVD Studies on the Treatment of Disease with the Interferon System - Levy..........................15-6

Z01 AI 00123-21 LVD Structure and Replication of Poxvirus DNA - Merchlinsky.....................................15-7

Z01 AI 00126-14 LVD Functional Analysis of Poxvirus Genome - DeFilippes......................................15-8

Z01 AI 00294-06 LVD Structure and Function of Adenovirus DNA - Rose...15-9

Z01 AI 00295-06 LVD Helper Factors Required for Expression of the Adeno-Associated Virus Genome - Rose..............15-10

Z01 AI 00296-06 LVD Characterization and Production of Parvovirus Proteins - Rose...................................15-11

Z01 AI 00297-06 LVD Mechanism and Regulation of Adeno-associated Virus DNA Replication - Rose.....................15-12

Z01 AI 00298-06 LVD Development of Vaccinia Virus as an Expression Vector - Moss 15-13

Z01 AI 00306-06 LVD Pathogenesis of Orthopoxvirus Infection - Buller..15-14

Z01 AI 00307-06 LVD Regulation of Vaccinia Virus Gene Expression - Moss..15-15

Z01 AI 00391-04 LVD Expression of Genes of Respiratory Viruses by Vaccinia Virus Recombinants - Moss................15-16

Z01 AI 00392-04 LVD Expression of Hepatitis B Virus Genes by Vaccinia Virus Recombinants - Moss........................15-17

Z01 AI 00393-04 LVD Expression of Malaria Genes by Vaccinia Virus Recombinants - Moss..............................15-18

Z01 AI 00416-04 LVD Recombinant Vaccines Against Retroviruses Associated with Leukemia and AIDS - Moss..........15-19

Z01 AI 00443-03 LVD Vaccinia Virus Growth Factor - Buller............15-20

Z01 AI 00445-03 LVD Mechanisms of Viral DNA Replication - Challberg...15-21

PHS-NIH
SUMMARY REPORT

ANNUAL REPORT OF THE LABORATORY OF VIRAL DISEASES, NIAID

October 1, 1986 to September 30, 1987

Dr. Bernard Moss
Chief, Laboratory of Viral Diseases

The Laboratory of Viral Diseases carries out a program of fundamental investigations on the molecular biology of viruses, the interactions of viruses with host cells, the pathogenesis of viral diseases, and host defense mechanisms. Current topics of research include: regulation of gene expression, mechanisms of DNA replication, virus growth factors, development of recombinant expression vectors, factors determining virus virulence, host resistance genes, targets of cell-mediated immunity, and genetic engineering of live recombinant vaccines. Emphasis is on vaccinia virus, ectromelia virus, herpes simplex virus, adenovirus, adenovirus associated virus and human immunodeficiency virus.

REGULATION OF VACCINIA VIRUS GENE EXPRESSION

Poxviruses are large DNA viruses that replicate in the cytoplasm of infected cells. In order to express and replicate their genomes outside of the nucleus, poxviruses must encode enzymes for these processes. This feature makes poxviruses, among which vaccinia virus is the prototype, unique for studying the regulation of transcription. During the past year, progress has been made in determining the signals used for initiation and termination of transcription of early vaccinia virus genes and the enzymes and factors needed to carry out these steps.

Mutagenesis studies demonstrated that the promoter region of the vaccinia virus thymidine kinase gene consists of a 30 base pair sequence just upstream of the RNA start site (Weir, Broyles, Davison and Moss).

A factor present in extracts of vaccinia virus was shown to bind specifically to the promoter region of early genes. Mutations in the promoter sequence which decrease transcription also decrease binding. Preliminary analysis indicates that this factor is a protein with a molecular weight of 130,000 (Yuen, Broyles, Davison and Moss).

Development of an accurate in vitro transcription system has made it possible to determine the signal used for termination of RNA chains. By constructing synthetic genes, the sequence TTTTTNT, in which N represents any nucleotide, was found to be necessary and sufficient to cause termination 50 base pairs downstream (Yuen and Moss).

Partially purified vaccinia virus RNA polymerase was shown to be incapable of termination. A complementation assay was developed to identify and isolate a termination factor. Surprisingly, the termination activity appears to reside in the enzyme that is also responsible for forming the cap structure at the 5' end of mRNA (Shuman, Broyles and Moss).

Vaccinia virus particles contain at least two DNA-dependent ATPases. The biological roles of these enzymes are not known. As a first step in learning their function, the gene for one of these enzymes, known as nucleoside triphosphate phosphohydrolase I, was identified. The deduced protein sequence indicated the presence of consensus ATP binding sites (Broyles and Moss).

REPLICATION OF VACCINIA DNA

Poxviruses provide a unique system for studying DNA replication. In vaccinia virus, the ends of the linear double-stranded DNA genome consist of hairpin structures that may resemble telomeres of eukaryotic chromosomes. The enzymes responsible for DNA synthesis appear to be virus encoded consistent with the cytoplasmic location of replication.

The DNA polymerase is being analyzed with regard to the location of aphidicolin-resistant mutations in order to map functional domains within the enzyme (DeFilippes).

Replicating vaccinia virus DNA consists of concatemers. The structure of the junction between unit genomes was shown to be a precise duplex copy of the viral terminal hairpin structure by cloning and sequencing the concatemeric junctions. An enzyme that can cleave the junction and ligate the ends of the DNA was partially purified (Merchlinsky and Moss).

ASSEMBLY OF VACCINIA VIRUS PARTICLES

Little is known about the assembly of the complex poxvirus particles. Previously, the drug rifampicin was shown to interfere with the assembly of vaccinia virions. Mutants that are resistent to this drug arise spontaneously. In order to learn the mechanism of resistance, the mutated gene was identified and sequenced. The deduced protein has a molecular weight of 62,000 (Baldick and Moss).

DEVELOPMENT OF VACCINIA VIRUS AS AN EXPRESSION VECTOR

Procedures have been developed for the insertion and expression of foreign genes in vaccinia virus. Originally we attached vaccinia virus regulatory sequences to the coding sequence of the foreign gene and depended upon transcription by the vaccinia virus RNA polymerase. More recently, we have achieved higher levels of expression by placing the RNA polymerase gene of bacteriophage T7 into vaccinia virus and attaching bacteriophage T7 regulatory sequences to the foreign coding sequence. In general, we now use the original method when the virus recombinants are to be injected into animals and the new method when high expression in tissue culture cells is desired (Fuerst and Moss).

Recombinant vaccinia viruses have been used to determine the targets of both the humoral and cell mediated immune response to viruses and other microorganisms. This is achieved by expressing a single foreign protein in a vaccinia virus vector. In collaboration with other laboratories, the immune response to influenza virus, respiratory syncytial virus, paramyxoviruses, rabies virus, herpes simplex virus, hepatitis B virus, Friend leukemia virus, human immunodeficiency virus, and Plasmodium falciparum have been obtained. In several cases it was shown that the major targets of humoral and cell mediated immunity do not reside on the same antigen. Frequently the internal proteins are as good or better cytotoxic T lymphocytes (CTL) targets than surface proteins. Nevertheless, protection of animals against viral disease is usually most effective when a humoral response is obtained to the surface proteins of the virus (Earl, Flexner, Hugin, Satchidanandam, and Moss).

STUDIES RELATED TO ACQUIRED IMMUNE DEFICIENCY DISEASE (AIDS)

Vaccinia virus recombinants expressing the envelope and gag genes of human immunodefiency virus (HIV) were constructed. Macaques vaccinated with the envelope recombinants developed antibodies directed against both the gp120 and gp41 envelope proteins. The sera of 3 of 4 animals neutralized HIV infectivity in vitro. However, this neutralization was largely HIV isolate specific. Recombinant vaccinia viruses were also used to prepare target cells to determine whether HIV seropositive individuals have CTL. Results indicated that the majority of seropositives have circulating CTL against both envelope and gag proteins (Earl and Moss).

VACCINIA VIRUS GROWTH FACTOR

Vaccinia virus has two copies of a gene that has amino acid sequence homology with the receptor binding site of epidermal growth factor and transforming growth factor alpha. In order to determine the biological role of the vaccinia virus growth factor, a mutant was constructed that has the active site deleted from both copies of the gene. Although able to grow well in tissue culture, the mutant virus has a markedly attenuated phenotype when injected intracranially in mice or intradermally in rabbits. On the chorioallantoic membrane of the chicken egg, wild-type vaccinia virus and mutant virus formed pocks with the same efficiency. The wild-type virus, however, induced marked proliferation of ectodermal and entodermal cells whereas the mutant did not. Initially, the proliferating cells had no virus antigen indicating that they were stimulated by secreted vaccinia growth factor. Eventually, the proliferating cells were infected and therefore the yield of wild-type virus was much higher than that of mutant virus. These data support an important role of the vaccinia growth factor in inducing cell proliferation (Buller and Moss).

PATHOGENESIS OF ORTHOPOXVIRUS INFECTIONS

In this project, attention has been focused on both the genetic basis of poxvirus virulence and host resistance to virus infection. A novel finding was made that anti-vaccinia virus primary CTL induction does not require L3T4+ cells. Further studies indicate that anti-vaccinia virus secondary CTL can be generated in vitro by a L3T4+ dependent or independent pathway at 3 to 4 weeks after priming. The finding of antigen specific but L3T4+ independent production of interleukin 2 by Ly2+ cells is unprecedented (Buller and Hugin).

The genetics of innate resistance to ectromelia virus in mice is being examined. Current experiments suggest that at least 2 mouse genes are involved in the resistance trait (Buller).

REPLICATION OF HERPES SIMPLEX VIRUS DNA

Herpes simplex viruses (HSV) are large DNA viruses that replicate in the nucleus of infected cells. Very little is currently known regrding the mechanism of HSV DNA synthesis. A transient complementation assay for identifying HSV genes required for DNA replication was developed. Using this assay, seven viral genes that are both necessary and sufficient to carry out authentic origin-dependent DNA replication were found. The sequence of each of these genes was determined. Antisera against 6 of the 7 deduced protein sequences were prepared and are being used to purify and investigate the replication proteins (Challberg, Hu, and Olivo).

REPLICATION OF ADENO-ASSOCIATED VIRUS DNA

Adeno-associated virus (AAV) is a member of the parvovirus family. The AAV genome is either a plus or a minus linear single-stranded DNA with partially palindromic inverted terminal repeats. The proposed mechanism of replication requires origin-specific single-strand cleavages in hairpin termini and at similar internal sites in concatemeric intermediates to yield unit length duplex templates from which progeny strands can be generated by displacement synthesis. To identify the predicted factors responsible for the site specific processing reactions, the products synthesized in cell-free extracts with DNA templates purified from AAV virions in 4 M guanidinium chloride-sucrose gradients were examined. A DNA-protein template component (P2) was obtained which directed synthesis of both unit-length hairpins and completely denaturable non-hairpin duplexes. Upon proteinase digestion, a fraction of the P2 molecules released terminal palindromic fragments suggesting that these fragments may be linked by a protein at the putative processing site (Chatterjee-Das, Sebring and Rose).

CHARACTERIZATION AND PRODUCTION OF AAV PROTEINS

AAV particles contain 3 primary capsid components of 90 (A), 72 (B) and 60 (C) kD. Aligned at their carboxy termini, they overlap so that B and C are contained in A and C is contained in B. In vitro translation studies indicate that both B and C can be made from a spliced 2.3 kb transcript by alternative use of a unique ACG initiation codon and an AUG codon. Protein A can be inefficiently synthesized from an unspliced 2.6 kb transcript but a more effective template may be generated by alternative splicing (Sebring, Wong, Muralidhar, Koczot and Rose).

Antibodies raised against synthetic peptides were use to detect and characterize six AAV2 non-structural proteins synthesized in vivo and in vitro. The transcripts for the proteins of 70, 26 and 22 kD are encoded by p5 promoter transcripts whereas the 50 and 42 kD proteins are directed by p19 transcripts (Sebring, Wong, Muralidhar, and Rose).

HELPER FACTORS REQUIRED FOR EXPRESSION OF THE AAV GENOME

Both adenovirus and herpes simplex virus can serve as helpers for the replication of AAV. A transient complementation assay was used to show that four XbaI clones plus the ICP4 gene of HSV-1 can support AAV replication. The XbaI D clone could be substituted with the individually cloned ICP0 and ICP27 genes. Continuation of these studies should reveal each of the HSV genes required (Mishra, Challberg, and Rose).

ADMINISTRATIVE CHANGES

Several major changes and additions were made to the Laboratory of Viral Diseases during the past year. The Molecular Structure Section of the Laboratory of Biology of Viruses headed by Dr. James Rose was transferred to LVD. The Genetic Engineering Section was created and Dr. Bernard Moss was appointed Section Head. Dr. Patricia Earl was designated head of the Biotechnology Unit within the Genetic Engineering Section. Dr. Moss resigned as Head of the Macromolecular Biology Section and Dr. Mark Challberg was appointed Acting Head. Drs. Jack Bennink and Jonathan Yewdell were recruited from the Wistar Institute to start a new Section of Viral Immunology.

HONORS AND AWARDS

Dr. Moss was elected a member of the National Academy of Sciences, received the PHS Distinguished Service Medal, the Solomon Berson Alumni Award in Basic Science from

New York University School of Medicine, and gave several named lectures including the Wallace P. Rowe lecture of the American Association of Laboratory Animal Science, the Morris F. Shaffer lecture of Tulane University, and the NIH lecture.

Members of LVD serve on the editorial boards of numerous scientific journals. Dr. Rose continues to serve as an associate editor of Virology and a member of the editorial board of Intervirology; Dr. Levy is on the boards of the Journal of Bioactive Polymers and the Journal of Biological Regulators and Homeostatic Agents; Dr. Moss is an associate editor of Virology and is on the boards of the Journal of Virology, Advances in Virus Research and the Journal of Biological Chemistry.

Members of LVD presented numerous lectures and seminars in this country and abroad.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00020-12 LVD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on the Treatment of Disease with the Interferon System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: H.B. Levy Section Head LVD, NIAID

COOPERATING UNITS *(if any)*
Dr. Meir Kende & Dr. Peter Canonico, USAMRIID; Dr. Andre Salazar, Walter Reed; Dr. Christopher Bever, V.A. Hospital, Memphis, Tenn.; Dr. James Talmadge, NCI; Dr. Samuel Baron, U. of Texas Med. School, Galveston, Texas.

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Molecular Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 1	PROFESSIONAL: 1	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
x☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Because I have neither a laboratory nor staff, I was not able to initiate new research last year. I have written three invited review chapters, and have helped develop two clinical protocols which I hope will be activated this coming year. In addition, analysis of data obtained in three different patient populations reveals that, as suspected, low doses of polyICLC are more effective as immune enhancing agents and are associated with much lower levels of adverse side effects. Low doses given intramuscularly are effective immune adjuvants, and can be given on an outpatient basis, with minimal side effects.

The nature of the changes in cell populations and activities strongly suggest that polyICLC should be tried in therapy of AIDS. The drug's enhancement of cell-associated and humoral antibodies to a variety of vaccines warrant a trial in association with vaccine to AIDS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00123-21 LVD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure and Replication of Poxvirus DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Merchlinsky	Staff Fellow	LVD, NIAID
Others:	B. Moss	Laboratory Chief	LVD, NIAID

COOPERATING UNITS *(if any)*

LPB, NIAID

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Genetic Engineering Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.1	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Poxviruses provide a unique experimental system for studying DNA replication. The ends of the linear double-stranded DNA genome consist of hairpin structures that may resemble telomeres of eukaryotic chromosomes. Enzymes and other proteins needed for DNA synthesis are encoded within the viral genome and replication occurs in the cytoplasmic compartment of infected cells. The DNA polymerase gene of vaccinia virus has been sequenced and the primary structure of the enzyme derived. Analysis of the DNA polymerase gene of a phosphonoacetate-resistant mutant virus revealed that a single nucleotide substitution results in drug resistance. This mutation may mark a part of the catalytic site of the enzyme.

Studies on the mechanism of formation of the hairpin telomeres are continuing. Evidence has been obtained that the ends are formed by resolution of near perfect palindromes separating unit genomes in large concatemers. An in vivo transfection assay for the resolution reaction was developed and evidence for a specific sequence requirement within the palindrome has been obtained. Resolution has been obtained in vitro using a partially purified enzyme fraction from vaccina virions.

Rifampicin is known to prevent assembly of DNA-containing vaccinia virus particles. The gene conferring drug resistance of a mutant vaccinia has been mapped and sequenced.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00126-14 LVD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Functional Analysis of Vaccinia Virus DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: F.M. DeFilippes Research Physicist LVD, NIAID

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Macromolecular Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 1.1	PROFESSIONAL: 1.0	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☑ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A vaccinia virus mutant which is resistant to the drug aphidicolin (AP) codes for an AP-resistant DNA polymerase. A change from a wild-type (WT) guanine to a thymine at position 2430 of the sequence of the DNA polymerase gene changes a leucine to a methionine in the polymerase and confers AP-resistance. The mutation was confirmed by site directed oligonucleotide mutagenesis of the WT DNA. To prepare the polymerase gene for the insertion of other mutations near position 2430 which might confer AP-resistance, a part of the WT gene, the Eco B segment, was cloned in a phagemid. Bgl I and Xho I restriction sites were inserted in the cloned Eco B segment at positions 2412 and 2514, respectively. Double-stranded oligonucleotides with sequences identical to the wild-type DNA except for single base changes can be inserted at the Bgl and Xho sites and the recombinant DNA tested for the ability to confer AP resistance. A phosphonoacetate resistance mutation was inserted at position 1537 to provide a means for selecting vaccinia virus with an altered B segment. Different mutagenic procedures were tested for their efficiency during the course of the work. The Bgl and Xho sites were inserted in the DNA by the Kunkel method which uses a bacterial strain that produces single-stranded template DNA containing uracil. This method, adapted for the phagemid system, had a 5% success rate for the creation of both the Bgl and Xho sites. When normal template DNA without uracil was used, the mutation frequency was tenfold less. The Eckstein method of mutagensis uses the restriction enzyme Nci, which will not cleave duplex DNA when one strand contains phosphorothioate nucleotides, but will nick a normal complementary strand. Initial results indicate a 90% success rate for the creation of the desired mutation. The conditions needed to increase the yield of the final DNA product used for the transformation reaction are under investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00294-06 LVD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure and Function of Adenovirus DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.A. Rose	Section Head	LVD, NIAID

COOPERATING UNITS *(if any)*
R. McPherson, M.D., Dept. Pathology, Georgetown University Hosp., Wash., D.C.;
D. Klessig, Waksman Inst., Piscataway, NJ;

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Molecular Structure Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.55	0.3	0.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Among objectives of these studies has been the application of physical, biochemical, and biological techniques to characterize the structure and functions of certain segments (i.e., the inverted terminal repeat) and genes of adenovirus (Ad) DNA. In earlier studies, we first identified and characterized the VA RNA gene/transcript and inverted terminal repeats in Ad DNA. We have continued to investigate the specifc regulatory functions of several early Ad genes, e.g., the VA and DNA-binding protein genes. Our results indicate that these latter genes are involved in the regulation of translation of certain viral mRNAs. Among methods used are gradient sedimentation, DNA cleavage with restriction endonucleases, gel electorphoresis, base sequence analysis, and DNA transfection.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00295-06 LVD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Helper Factors Required for Expression of the Adeno-Associated Virus Genome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	J.A. Rose	Section Head	LVD, NIAID
Others:	L. Mishra	Visiting Associate	LVD, NIAID

COOPERATING UNITS *(if any)*

M. Challberg	Senior Staff Fellow	LVD, NIAID

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Molecular Structure Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 1.50	PROFESSIONAL: 1.25	OTHER: 0.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The main objectives of this project are (1) to define where and how each required helper virus factor regulates expression of defective human paravovirus (AAV) genomes and (ii) to relate these findings to their respective roles in the replication of the helper virus (adenoviruses, herpesviruses) themselves as well as to potentials for selective interference with viral infection. We previously mapped the adenovirus genes required for AAV replication and continue to investigate their specific helper functions. Similar studies are in progress with herpes simplex viruses. Among methods used are specific immunofluorescence, cleavage of DNA with restriction endonucleases, DNA cloning, gel electrophoresis, blot-hybridization analyses and DNA transfection of both simian and human cell lines.

PHS 6040 (Rev. 1/84) GPO 914-918

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00296-06 LVD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characterization and Production of Parvovirus Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.A. Rose	Section Head	LVD, NIAID
Others:	E. Sebring	Research Chemist	LVD, NIAID
	K. Wong	Medical Staff Fellow	LVD, NIAID
	S. Muralidhar	Visiting Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*
C.W. Anderson, Brookhaven National Laboratory, Upton, NY 11973

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Laboratory of Viral Diseases

SECTION
Molecular Structure Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 4.85	PROFESSIONAL: 3.1	OTHER: 1.75

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The main objectives of these studies are (1) to identify and characterize all proteins that are specified by the defective human parvoviruses (AAV) and to determine similarities and differences with autonomous parvovirus proteins (2) to define the mechanism(s) by which the AAV proteins arise and (3) to define specific functions of the AAV proteins. We have identified several AAV non-structural proteins which were previously undetected. At least one of these proteins is necessary for viral DNA replication. Post-translational processing does not account for production of any AAV structural proteins although they share large segments of sequences-in-common. It is now clear, however, that these proteins originate form independent in-frame initiations. The mechanism that regulates translation of AAV proteins is of fundamental interest, and we have now shown that one AAV structural protein is initiated by a codon not known previously to act as an initiation codon in eukaryotes. Furthermore, our current findings support a "scanning mechanism" in the translational expression of polycicstronic eukaryotic mRNAs. Among methods used are site-directed mutagenesis, affinity chromotography, gel electrophoresis, in vitro translation of viral RNA, electrophoretic and HPLC analyses of V8 protease and tryptic peptides and aminoterminal sequencing of purified polypeptides.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00297-06 LVD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Mechanism and Regulation of Adeno-associated Virus DNA Replication

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.A. Rose	Section Head	LVD, NIAID
Others	E. Sebring,	Research Chemist	LVD, NIAID
	S. Chatterjee-Das	Visiting Associate	LVD, NIAID

COOPERATING UNITS *(if any)*

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Laboratory of Viral Diseases

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH, Behtesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.25	1.5	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The primary objective of this project is to define molecular and biochemical mechanisms involved in eukaryotic DNA synthesis. To approach this problem, we are investigating adeno-associated virus (AAV) DNA replication in both in vivo and in vitro systems. Current results show that AAV DNA synthesis can be initiated in vitro in cell-free extracts and that replicating forms that correspond to those identified in vivo can be synthesized with either endogenous or exogenously added templates. Recently, we have identified two DNA-associated proteins that may play an important role in the required processing of self-primed unit lenth hairpin and concatemeric intermediates of DNA replication. The observed mode of AAV DNA replication may serve as a model for telomere replication in eukaryotes. Among methods used are affinity chromatography, gel electrophoresis, DNA sequence analysis and restriction cleavage of DNA molecules.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00298-06 LVD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Vaccinia Virus as an Expression Vector

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Moss	Laboratory Chief	LVD, NIAID
Others	F.G. Falkner	Guest Researcher	LVD, NIAID
	C. Flexner	Medical Staff Fellow	LVD, NIAID
	T. Fuerst	Staff Fellow	LVD, NIAID
	G. Kotwal	Visiting Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.95	2.4	1.45

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Procedures have been developed for the use of vaccinia virus as a eukaryotic expression vector. A chimeric gene is formed by ligating vaccinia virus transcription regulatory signals to a foreign protein coding sequence. Homologous recombination is used to insert the chimeric gene into a non-essential region of the vaccinia virus genome. To facilitate the formation and isolation of recombinant virus, new plasmid vectors have been constructed that have stronger promoters and multiple cloning sites and which direct the insertion of the chimeric gene together with the E. coli B-galactosidase gene into the thymidine kinase locus. Recombinant virus is then selected on the basis of the thymidine kinase negative phenotype and/or staining with a B-galactosidase indicator dye. The recombinant viruses produced in this manner are stable and have a wide host range for tissue culture cells and animals. This system has been used to express genes from a variety of infectious agents including herpes simplex virus type 1, hepatitis B virus, influenza virus, vesicular stomatitis virus and rabies virus. Animals vaccinated with each of the above recombinants were protected against challenge with the corresponding virus.

A new eukaryotic expression system based on co-infection with two recombinant viruses one synthesizing bacteriophage T7 RNA polymerase and the other containing a foreign gene with bacteriophage T7 regulatory signals has been developed. This new system is more efficient than previous vaccinia virus expression systems that rely exclusively on vaccinia transcriptional signals.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00306-06 LVD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pathogenesis of Orthopoxvirus Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.M.L. Buller	Visiting Scientist	LVD, NIAID
Others:	A. Hugin	Guest Researcher	LVD, NIAID
	C. Duarte	Bio. Lab. Tech. (Micro)	LVD, NIAID

COOPERATING UNITS *(if any)*
LIP, NIAID (H.C. Morse, III, R. Yetter, and K. Holmes); Cancer Immunology Branch, NCI (Dr. G. Shearer, Dr. A. Singer, Dr. T. Mizuochi); U. of Connecticut (T. Frederickson); Dr. D. Pickup, Duke University.

LAB/BRANCH
Laboratory of Viral Disease

SECTION
Macromolecular Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	0.8	0.8

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In this project we focussed our studies on the genetic basis both of orthopoxvirus virulence and of host resistance to virus infection. The acquired knowledge should contribute towards the development of safe, effective, recombinant vaccinia virus vaccines for animal and human use. An insertional inactivation mutagenesis system suitable for screening large regions of the ectromelia virus genome has been developed and is being used to analysis a 7.3 kb fragment of DNA for genes important for virus virulence in vivo. Our observation reported last year that anti-vaccinia virus primary CTL induction did not require L3T4$^+$ cells was extended through the use of secondary in vitro mixed lymphocyte reaction (MLRs). These studies indicated that anti-vaccinia virus secondary CTL responses can be generated by a L3T4$^+$ dependent or independent pathway at 3-4 weeks after priming. Antigen specific IL-2 production was detected in the MLR culture which showed vaccinia virus specific CTL but lacked L3T4$^+$ cells. This is the first report of L3T4$^+$ independent IL-2 production by Ly2$^+$ cells in response to virus antigen. A requirement of L3T4$^+$ cells for the generation of CTL in MLR, however, was noted in mice which had been primed 8 weeks or more prior to testing. Using the cross-intercross system, the A strain of (disease susceptible) mouse was crossed with a resistant C57BL/6J mouse in order to generate a mouse strain A.B6-Rmp (resistance to mousepox) congenic for the resistance loci. This will aid in studying the non-H2 innate resistance genes important in controlling virus spread.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00307-06 LVD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Regulation of Vaccinia Virus Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Moss	Laboratory Chief	LVD, NIAID
Others:	A.J. Davison	Visiting Associates	LVD, NIAID
	S. Broyles	Staff Fellow	LVD, NIAID
	L. Yuen	Visiting Fellow	LVD, NIAID
	S. Shuman	Medical Staff Fellow	LVD, NIAID
	C. Wright	Guest worker	LVD, NIAID
	N. Stomatos	NRC Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Genetic Engineering Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.8	3.6	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Vaccinia virus has a genome of about 185,000 base pairs that encodes approximately 200 polypeptides. These genes are expressed within the cytoplasm in a coordinated fashion so that some polypeptides are made before and others after DNA replication. The enzymes responsible for expression of early genes are packaged within the infectious virus particle. The aim of this project is to determine the mechanisms regulating transcription. This involves both an analysis of the enzymes and protein factors and the DNA signals.

The promoter regions of early and late genes have been localized within 30 base pairs upstream of the RNA start sites. The termination signal of early genes has been defined as TTTTTNT, where N can be any nucleotide, and is located about 50 base pairs upstream of the actual site of termination.

An extract of vaccinia virus particles that can accurately initiate and terminate transcription has been developed. The RNA polymerase has at least 8 virus-encoded subunits which have been mapped on the genome. The largest subunit has been shown to have extensive sequence homology with the large subunit of eukaryotic RNA polymerase. A factor that binds specifically to promoter regions of early genes has been isolated. A transcription termination factor that copurified with capping enzyme has been characterized. A DNA-dependent ATPase, with unknown biological role has been mapped and the protein sequence deduced.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00391-04 LVD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression of Genes of Respiratory Viruses by Vaccinia Virus Recombinants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Moss	Laboratory Chief	LVD, NIAID
Others:	C. Flexner	Medical Research Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*
University of Cambridge; the Wistar Institute; LID, NIAID; Northwestern University

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Genetic Engineering Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Development of effective vaccines against respiratory viruses requires a thorough understanding of the immunological response to individual proteins. For this reason panels of recombinant vaccinia viruses expressing individual influenza A, respiratory syncytial (RS), and paramyxovirus SV5 proteins were constructed. In the case of influenza, a complete set of 9 recombinant viruses were made. For respiratory syncytial virus and SV5, recombinants expressing the 2 envelope proteins were prepared. In all cases the synthesized polypeptides closely resembled the natural ones with regard to electrophoretic mobility, antigenicity, and intracellular transport. The recombinant vaccinia virus expressing HA was most effective at protecting mice against respiratory influenza infection. Cottons rats were protected against RS virus most effectively with the recombinant expressing the F protein although considerable protection also was obtained with the G recombinant. By contrast, cottons rats were better protected from SV5 infection with the vaccinia expressing HN than with vaccinia expressing F, although the latter induced even higher levels of neutralizing antibody. The ability of all of the recombinant vaccinia viruses to induce cell mediated immunity and to serve as cytotoxic T cell targets was measured.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00392-04 LVD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression of Hepatitis B Virus Genes by Vaccinia Virus Recombinants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Moss	Laboratory Chief	LVD, NIAID
Others:	K.C. Cheng	Guest Researcher	LVD, NIAID
	T. Fuerst	Staff Fellow	LVD, NIAID

COOPERATING UNITS *(if any)*
Georgetown University, Scripps Clinic

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Genetic Engineering Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 0.4 0.2	PROFESSIONAL: 0.2	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hepatitis is a serious world-wide health problem. Approximately 200 million people are chronically infected with hepatitis B virus, and large numbers of deaths are attributed to fulminant hepatitis, cirrhosis, and hepatocellular carcinoma. Although an effective subunit vaccine has been produced, limitations in supply and expense have prevented its global use. As an alternative, we are trying to construct a live recombinant hepatitis B vaccine. The gene for hepatitis B virus surface antigen has been engineered and inserted into the genome of vaccinia virus. The recombinant vaccinia virus is stable and expresses the hepatitis virus protein. The latter is glycosylated, assembled into particles and transported through the plasma membrane of infected cells. Rabbits vaccinated with the recombinant virus produced a high and sustained specific antibody response. Vaccination of chimpanzees resulted in priming of the immune system and protection against clinical hepatitis upon subsequent challenge with hepatitis B virus. Recombinant vaccinia viruses that express pre-S as well as S antigens are being evaluated.

Additional recombinant vaccinia viruses that express genes of hepatitis delta virus, a naturally occurring defective virus of man have also been made.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00393-04 LVD

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Expression of Malaria Genes by Vaccinia Virus Recombinants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Moss	Laboratory Chief	LVD, NIAID
Others:	V. Satchidanandam	Guest Researcher	LVD, NIAID

COOPERATING UNITS *(if any)*

New York University, LPD, NIAID

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland, 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Malaria remains a serious global health problem for which there is no effective vaccine. Previous studies indicate that animals can be immunized with inactivated sporozoites. The gene coding for the circumsporozoite (CSP) antigen of the malaria parasites Plasmodium falciparum and P. knowlesi have been inserted into the vaccinia virus genome under the control of a defined vaccinia virus promoter. Tissue culture cells infected with the recombinant synthesized polypeptides that reacted with monoclonal antibody against the repeat epitopes of P. falciparum or P. knowlesi CSP proteins. Mice and rabbits inoculated intradermally with the recombinant viruses produced antibody that bound to sporozoites. The recombinant viruses are being used to help define the important T-cell epitopes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00416-04 LVD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Recombinant Vaccines Against Retroviruses Associated with Leukemia and AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Moss	Laboratory Chief	LVD, NIAID
Others:	F. G. Falkner	Guest Researcher	LVD, NIAID
	S. Merli	Guest Researcher	LVD, NIAID
	C. Flexner	Medical Research Fellow	LVD, NIAID
	E. Berger	Expert	LVD, NIAID

COOPERATING UNITS *(if any)*
LPVD, NIAID; LIR, NIAID; LTCB, NCI; Division of Virology, OBRS, FDA; Massachusetts General Hospital

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Genetic Engineering Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.35 0.85	1.5	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human X subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Summary of Work

Retroviruses, long associated with leukemia and sarcoma of animals, have recently been implicated as the etiological agents of human T cell leukemia and human acquired immune deficiency disease (AIDS). The identification of these agents makes it possible to consider various ways of prevention. The most promising approach is development of a vaccine that could be administered to individuals at risk. We have been investigating two ways of making vaccines: use of a live virus vector expressing human immunodeficiency virus (HIV) genes and synthesis of a subunit protein by recombinant DNA methods. For the first, recombinant vaccinia viruses that express envelope and other HIV genes have been prepared and shown to be immunogenic. A high expression system using bacteriophage T7 RNA polymerase in a vaccinia virus vector has been used for synthesis of HIV proteins in mammalian cell culture.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00443-03 LVD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)
Vaccina Virus Growth Factor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	R.M.L. Buller	Visiting Scientist	LVD, NIAID
Others:	B. Moss	Laboratory Chief	LVD, NIAID
	C. Duarte	Bio. Lab. Tech. (Micro)	LVD, NIAID

COOPERATING UNITS (if any)

ONCOGEN (D. Twardzik); U. Connecticut (T. Frederickson)

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Macromolecular Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.3	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Vaccinia virus has two copies of a gene which has amino acid sequence homology with the receptor binding site of epidermal growth factor (EGF) and transforming growth factor (TGF-α). A 25 Kilodalton glycoprotein (vaccinia virus growth factor [VGF] that can bind to EGF receptors) has been purified from media of virus infected BS-C-1 cells. When a mutant virus (VGF^-) was constructed by removing both copies of the sequence encoding the putative EGF receptor binding site, the EGF receptor binding activity in infected cells was abolished. Inocuations of the VGF^- mutant into BALB/cByJ mice by the intracranial route resulted in an LD_{50} which was 100-fold higher than the wild-type (WT) virus. A similar attenuated phenotype was observed with the mutant on intradermal inoculation of the skin of a New Zealand white rabbit. Both WT and VGF^- mutant virus infected with similar efficiencies BS-C-1, A431 and Swiss 3T3 cell monolayers, even though the density of EGF receptors varied considerably among the cell lines. It was also shown that an anti-EGF receptor MAB 528 reduced the plaquing efficiency of both WT and VGF^- mutant virus on A431 cell monolayers only slightly and to the same extent. These data support the notion that VFG binding to the EGF receptor is not required for virus entry into host cells. Using non-proliferating, Swiss 3T3 cells, WT virus was shown to replicate more efficiently than VGF^- mutant, and suggested a role for VGF in the enhancement of virus replication under conditions which did not support cell growth. On the CAM of the egg, both WT and mutant virus formed pocks with approximately the same efficiency. The WT, but not the mutant, induced extensive ectoderm and endoderm proliferation. The proliferating cells were shown to be initially uninfected. WT lesions were on the whole slightly larger than the mutant, and contained greater amounts of infectivity and antigen. Both viruses induced a similar inflammatory response in the host which was observed at 40 hours post innoculation. These results show that in vivo VGF can induced non-dividing cells to proliferate dramatically which increases the rate of virus replication and spread within the lesion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00445-03 LVD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanisms of Viral DNA Replication

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mark D. Challberg	Senior Staff Fellow
Others:	Carol Wu	Staff Fellow
	Paul Olivo	Medical Fellow

COOPERATING UNITS *(if any)*
Institute of Virology, Glasgow, Scotland; J.A. Rose, Molecular Structure Section, Laboratory of Viral Diseases, NIAID

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Macromolecular Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 3.35	PROFESSIONAL: 2.25	OTHER: 1.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Herpes simplex virus is a useful model for studying the mechanisms involved in DNA replication in eukaryotic cells. Our current efforts are directed toward studying this process with purified proteins. We have developed a transient complementation assay for identifying viral genes required for viral DNA replication. Using this assay, we have found seven viral genes which are both necessary and sufficient to carry out authentic origin-dependent DNA replication. The sequence of each one of these seven genes has been determined. Using the predicted amino acided sequence of the products of each of these genes as a starting point, we have raised rabbit antisera against six individual replication proteins. The antisera have been used in immunoblot experiments to characterize the proteins in extracts of HSV-infected cells. We are currently using the antisera as assays for the purification of each of the required gene products. Current experiments are aimed at determining the biochemical function of the purified proteins.

PHS 6040 (Rev. 1/84) GPO 914-918

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LABORATORY OF MICROBIAL STRUCTURE AND FUNCTION
Rocky Mountain Laboratories
Hamilton, Montana
1987 Annual Report
Table of Contents

Z01 AI Project Number		Page
Summary		16-1
00065-13	Antigens and Classification of Rickettsiae -- Anacker (terminated)	16-6
00193-08	Gonococcal Surface Components: Structure and Function -- Swanson	16-7
00216-07	Immunology of Chlamydial Surface Antigens -- Caldwell	16-8
00234-06	Biology of Intracellular Parasitism -- Hackstadt	16-9
00235-05	Studies on Nonprotein Surface Constituents of *Neisseria gonorrhoeae* -- Hitchcock (terminated)	16-10
00413-04	Molecular Genetics of *Chlamydia trachomatis* -- Nano	16-11
00441-03	*Rickettsia rickettsii* Antigens -- McDonald	16-12
00442-03	Immunobiology of Guinea Pig Inclusion Conjunctivitis -- Watkins	16-13

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Annual Report
Laboratory of Microbial Structure and Function
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1986, to September 30, 1987

RESEARCH HIGHLIGHTS

Research in LMSF examines bacterial surface components that play roles in the pathogenesis and virulence of selected pathogenic bacteria. Both protein and nonprotein surface constituents of gram-negative bacteria are prime targets for study since they mediate many critical interactions between microbe and host; such bacterial components also have potential as immunotherapeutic and diagnostic reagents. The current research by LMSF staff concentrates on chlamydiae, gonococci, spotted fever rickettsiae, and Q-fever organisms (*Coxiella burnetii*).

Chlamydiae: LMSF scientists in the Chlamydial Diseases Section led by H. Caldwell have identified several important aspects of the major outer membrane protein (MOMP) of chlamydiae. This surface protein, whose structure differs in the various chlamydial serotypes, is a major serotype determiner of chlamydiae and the protection that follows chlamydial infections of guinea pig and subhuman primate conjunctivae is limited to those organisms with the homologous MOMP serotype. Recent studies by Y-X. Zhang and Caldwell implicate MOMP as the target for monoclonal antibodies that neutralize chlamydial infectivity, in vitro. This neutralization coincides with reduction of the adherence of chlamydiae to host cells as shown by experiments of Zhang, Su Hua, and Caldwell that utilize an extensive set of anti-MOMP monoclonal antibodies and partial proteolysis of MOMP in situ. The LMSF chlamydiae group has made impressive progress in defining those portions of MOMP that bear "immunoprotection-related" epitopes. These advances by T. Joseph, Zhang, Hua, F. Nano, W. Baehr, and Caldwell relied on molecular cloning and sequencing the MOMP genes from chlamydiae of several different serovars and epitope mapping based on those nucleotide sequences as well as on demonstrated antigenicities of proteins expressed in lambda gt11 by whole MOMP genes or partially deleted MOMP genes. Those studies identified type-, subspecies-, and species-specific epitopes of MOMP. Also tentatively identified by these studies were T-lymphocyte recognition sites of MOMP as well as portions of MOMP that are recognized by B-lymphocytes. Studies by the LMSF chlamydiae group had shown previously that primary conjunctival infections in either guinea pigs or cynomolgus monkeys led to development of ocular hypersensitivity directed toward a component common to all chlamydiae. This ocular hypersensitivity produces an intense inflammation in conjunctivae of the previously infected, recovered animals that, coincidentally, are immune to conjunctival infection by homologous serotype chlamydiae; the inflammatory reaction is elicited when either whole chlamydiae or their Triton X-100 extracts are introduced onto the conjunctivae of these animals. Those studies by N. Watkins, A. Moos, and Caldwell indicate that similar ocular hypersensitivity develops after primary chlamydial infections of vaginal or gastrointestinal sites; hypersensitivity also occurs in the guinea pig oviduct after a primary chlamydial infection in that locale. Ocular hypersensitivity is elicited by chlamydial components that are synthesized in large amounts by chlamydiae-infected epithelial cells that are treated with penicillin to arrest the differentiation and development cycle typical of chlamydiae that grow obligately in host cells. Caldwell demonstrated that chlamydiae-infected, penicillin-treated tissue culture cells elaborate the hypersensitivity-evoking component as assayed in eyes of guinea pigs previously infected with and recovered from primary chlamydial conjunctivitis. Watkins, Moos, and Caldwell found that if penicillin is applied to a guinea pig's conjunctivae that harbor an acute, primary chlamydial infection, corneal opacity ensues promptly and then wanes when penicillin therapy ceases; the histopathologic changes in these opacified corneas mimic those of human trachoma, and they argue for this being a reasonably apt animal model of trachoma. Two inbred strains of guinea pigs show strikingly different clinical courses in their primary conjunctival infections with chlamydiae and in their post-infectious development of hypersensitivity as found by Watkins. The chlamydial component responsible for evoking ocular hypersensitivity has been tentatively identified by R. Morrison and Caldwell as a heat-labile cysteine-rich polypeptide (M_r=57 kD) that can be harvested from culture fluids of penicillin-treated, chlamydiae-infected tissue culture cells. Nano collaborated with H. Brade (Borstel, FRD) to define the chemical structure of LPS that is modified to contain an epitope encoded by a gene cloned from C. trachomatis. This chlamydial gene encodes a glycosyl transferase whose synthetic product is a chlamydiae-genus LPS epitope even when it is expressed in E. coli or in salmonella of assorted chemotypes. This LPS epitope is common to all chlamydiae and its synthesis-encoding gene may prove useful for development of a diagnostic reagent.

Gonococci: Two surface components of gonococci--pili and outer membrane protein II (P.II)--were studied by J. Swanson. He found that nonreciprocal homologous recombination (gene conversion) between the complete, expressing pilin gene and a silent, partial pilin gene accounts for both antigenic and reversible phase changes of pili in this bacterium. This chromosomal pilus expression system utilizes gene conversion to spawn an almost infinite number of different pilin polypeptides; this was clearly seen when gonococci emanating from experimentally infected males were examined and compared to input organisms as to their pilin mRNA sequences. The high degree of structural variety observed among these gonococci included a region of pilin polypeptides previously thought to be conserved and, therefore, a vaccine candidate. Expression of P.II by gonococci emanating from infected volunteers was also examined; virtually all organisms reisolated from the experimental gonorrheal infections expressed one or more P.II polypeptides in contrast to the absence of such P.II expression by input bacteria. On/Off changes of P.II expression by gonococci is due to recombination-independent nucleotide sequence changes in that region of each P.II gene that lies between its translation initiation site and its mature polypeptide's ORF; this region is rich in direct pentameric nucleotide repeats (CTTCT). Several P.II genes have been cloned by van der Ley who is studying control of their encoded proteins in E. coli. P. Hitchcock continued her examination of LPS and of the H.8 antigen present in gonococci. Variation of LPS structure was demonstrated for input versus reisolated gonococci that were obtained from experimental gonorrheal infections of male volunteers. W. Baehr and Hitchcock completed the nucleotide sequence of a cloned gene that encodes a highly antigenic polypeptide portion of the H.8 moiety. This 6.9-kD polypeptide has novel structure as it contains 14 tandemly arranged imperfect pentameric (amino acid) repeats; its amino acid composition is, accordingly, unusual (alanine, proline, glutamic acid-rich, no aromatic amino acids nor methionine). Lipid appears to account for a sizeable fraction of the final H.8 product in which the 6.9-kD polypeptide comprised the antigenically dominant portion.

Coxiella burnetii and *Rickettsia rickettsii*: Examination of *C. burnetii* LPS by *T. Hackstadt* led to discovery of several distinct LPS variants and correlation of LPS form with virulent vs. avirulent "phases" of this organism. Recent studies disclose that different LPS forms of this obligate intracellular parasite correlate with fever production in experimental animal infections. One of these LPS variants typifies *C. burnetii* from individuals with chronic Q-fever endocarditis; but use of a rabbit model for endocarditis by *A. Moos*, *S. Vishwanath*, and *Hackstadt* has yet to identify a special capability of this LPS variant in causing such endocarditic lesions. *Vishwanath* and *Hackstadt* found that LPS variants of *C. burnetii* differ in their liabilities to killing by serum complement components; their relative susceptibilities to such killing were correlated with either differences in binding the early complement C3b component or with failing to support activation late complement components. These studies aid explanation of lack of virulence for some *C. burnetii* variants (phase II). *E. coli* that contains a gene cloned from *R. rickettsii* stimulates production of specific antibodies that protect immunized guinea pigs against infectious challenge with spotted fever rickettsiae. These studies by *G. McDonald* provide additional evidence that the protein (M_r=155 kD) encoded by this cloned *R. rickettsii* gene may have utility as a vaccine against Rocky Mountain spotted fever.

ADMINISTRATIVE REPORT

Personnel changes during the past year include arrival of Dr. Seth Pincus, Expert Consultant from University of Utah, Dr. Carol Horgan, Senior Staff Fellow from University of Washington, and two Visiting Fellows, Dr. Peter van der Ley from Utrecht, The Netherlands, and Dr. Robert Belland from Victoria, BC, Canada, and departure of Dr. Penny Hitchcock to University of Tennessee, Memphis. Visiting Researchers Franco Menozzi and Catherine Dejaiffe, Mons University, Mons, Belgium, spent nine months in LMSF, and Dr. Carl Frasch, Center for Drugs and Biologics, Bethesda, MD, spent three months in LMSF. Summer students included: Michael Jasumback and James Sola (Carroll College), Nancy Joste (University of New Mexico), and Lisa Milch (Montana State University). Seminars were given by visitors as follows: Milan Blake (The Rockefeller University, New York, NY); Troy Couch (Thomas Jefferson University, Philadelphia, PA); Deborah Cutter (North Carolina State University, Raleigh, NC); Harvey Eisen (Fred Hutchinson Cancer Research Center, Seattle, WA); Rachael Demarco de Hormaeche (University of Cambridge, Cambridge, England); Dorothy Patton (University of Washington, Seattle, WA); Howard Raff (Genetic Systems, Inc., Seattle WA); and John Swindle (Fred Hutchinson Cancer Research Center, Seattle, WA).

J. Swanson received the NIH Director's Award

Journal Editorial Boards:

J. Swanson - *Infection and Immunity*
P. Hitchcock - *Infection and Immunity* and *Journal Bacteriology*

Manuscripts were reviewed by LMSF staff for the following journals: *Canadian Journal of Microbiology*, *Infection and Immunity*, *Journal of Bacteriology*, *Journal of Biological Chemistry*, *Journal of Clinical Investigation*, *Journal of Clinical Microbiology*, *Journal of General Microbiology*, *Journal of Immunology*, *Journal of Infectious Diseases*, *Proceedings of the National Academy of Sciences, USA*, *Science*, and *Sexually Transmitted Diseases*.

Professional Posts:

J. Swanson - Member, Microbiology and Infectious Diseases Research Committee, NIAID

O. Barrera - Promotion Advisory Committee, NIAID

H. Caldwell - Faculty Affiliate, Department of Microbiology, University of Montana, Missoula, MT
Promotion Advisory Committee, NIAID

T. Hackstadt - Consultant Member, Food and Drug Administration, Orphan Products Development Initial Review Group, Rockville, MD

Invited Lectures and Participation in Meetings and Symposia:

J. Swanson - UCLA Symposium on Bacteria-Host Cell Interaction, Park City, UT
Gordon Conference on Molecular Mechanisms of Microbial Adhesion, Newport, RI
The Rockefeller University, New York, NY
University of Memphis, Memphis, TN
University of Texas Health Science Center, San Antonio, TX
University of Texas Southwest Medical Center, Dallas, TX

W. Baehr - Kernforschungsanlage, Juelich, West Germany

H. Caldwell - Edna McConnell Clark Foundation Workshop on Trachoma Immunology and Pathogenesis, Woods Hole, MA

T. Hackstadt - American Society for Microbiology, Atlanta, GA
University of Alberta, Edmonton, Alberta, Canada
University of Pittsburgh, Pittsburgh, PA
University of Hawaii, Honolulu, HI
University of Idaho, Moscow, ID

P. Hitchcock - American Society for Microbiology, Atlanta, GA
Stanford University School of Medicine, Stanford, CA
Bristol-Myers Corp., Wallingford, CT
Boston University, Maxwell Finland Laboratory, Boston, MA
University of Alberta, Faculty of Medicine, Edmonton, Alberta, Canada
Walter Reed Army Institute for Research, Washington, DC
National Institute of Allergy and Infectious Diseases, Bethesda, MD
Bureau of Biologics, Washington, DC
University of Tennessee, Memphis, TN

S. Hua - American Society for Microbiology, Atlanta, GA

T. Joseph - American Society for Microbiology, Atlanta, GA

G. McDonald - American Society of Rickettsiology, Williamsburg, VA
Microbial Surfaces: Determinants of Virulence and Host Responsiveness, Oakland, CA
Washington State University, Pullman, WA

A. Moos - UCLA Symposium on Bacteria-Host Cell Interaction, Park City, UT

R. Morrison - Edna McConnell Clark Foundation Workshop on Trachoma Immunology and Pathogenesis, Woods Hole, MA
American Society for Microbiology, Atlanta, GA

F. Nano - Boehringer Mannheim, Indianapolis, IN
University of Idaho, Moscow, ID
University of Alberta, Edmonton, Alberta, Canada

S. Vishwanath - American Society for Microbiology, Atlanta, GA

N. Watkins - Edna McConnell Clark Foundation Workshop on Trachoma Immunology and Pathogenesis, Woods Hole, MA
UCLA Symposium on Bacteria-Host Cell Interaction, Park City, UT
American Society for Microbiology, Atlanta, GA

Y-X. Zhang - Edna McConnell Clark Foundation Workshop on Trachoma Immunology and Pathogenesis, Woods Hole, MA
American Society for Microbiology, Atlanta, GA

Other Activities:

H. Caldwell - Reviewed research grants for Medical Research Council, Canada, and The Edna McConnell Clark Foundation, New York, NY

T. Hackstadt - Reviewed research grant for National Science Foundation, Washington, DC

P. Hitchcock - Reviewed research grants for British Columbia Health Care Research Foundation, British Columbia, Canada, and Department of Energy, Washington, DC

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00065-13 LMSF

PERIOD COVERED

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Antigens and Classification of Rickettsiae

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. L. Anacker	Research Microbiologist	LMSF, NIAID
Others:	G. A. McDonald	Staff Fellow	LMSF, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Dr. Robert Anacker retired August 1986, therefore this project is terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00193-08 LMSF

PERIOD COVERED
October 1, 1986, to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gonococcal Surface Components: Structure and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Swanson	Chief	LMSF, NIAID
Other:	P. van der Ley	Visiting Fellow	LMSF, NIAID
	R. Belland	Visiting Fellow	LMSF, NIAID
	O. Barrera	Microbiologist	LMSF, NIAID
	K. Robbins	Microbiologist	LMSF, NIAID
	D. Corwin	Bio. Lab. Tech.	LPB, NIAID

COOPERATING UNITS *(if any)*
Michael Koomey, Rockefeller University, New York, NY
Milan Blake, Rockefeller University, New York, NY
John Boslego, WRAIR, Washington, DC

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.75	1.65	3.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Gonococci (Gc) rely on their surface constituents for those interactions with a human host that promote gonorrheal infection. Pili are thought to anchor Gc to human mucosal sites. But in vivo Gc rapidly and regularly change from synthesizing one pilin subunit to making another. This pilus subunit variation relies on recombinational interactions between the Gc genome's single complete pilin gene and one or another of its partial pilin genes. These "gene conversion" events lead to pilin structural diversity when either different partial pilin genes or different segments of an individual partial gene undergo nonreciprocal recombinational insertion into the complete pilin gene. Marked structural diversity occurs in pilin subunits of Gc causing gonorrheal infections in male volunteers; such amino acid sequence and antigenic changes occur within the carboxy-terminal one half of the pilin molecule and includes regions previously thought to be invariant. Outer membrane protein II (P.II) of Gc has a less clearly defined role in pathogenesis of gonorrhea, but one or more of these surface-exposed polypeptides is regularly expressed by Gc recovered from male urethral sites; these proteins likely aid colonization by virtue of the inter-Gc aggregation they effect. Control of each P.II is exerted by recombination-independent changes in the sequence of its respective gene. In Gc recovered from experimentally infected males, one or two P.II species were regularly expressed, and the Gc from each male preferentially expressed some, but not other, P.II moieties.

LPB

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00216-07 LMSF

PERIOD COVERED
October 1, 1986, to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunology of Chlamydial Surface Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	H. D. Caldwell	Research Microbiologist	LMSF, NIAID
Others:	F. E. Nano	Senior Staff Fellow	LMSF, NIAID
	Y.-X. Zhang	Visiting Fellow	LMSF, NIAID
	Su Hua	Visiting Fellow	LMSF, NIAID
	W. B. Baehr	Senior Staff Fellow	LMSF, NIAID
	N. G. Watkins	Staff Fellow	LMSF, NIAID

COOPERATING UNITS *(if any)*
Hugh Taylor, Johns Hopkins University School of Medicine, Baltimore, MD, and Richard Morrison and Theresa Joseph, Foundation for Advanced Education in Science

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION
Chlamydial Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
5.65	3.5	2.15

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The overall goal of this project is to develop a recombinant or synthetic vaccine for the prevention and/or control of Chlamydia trachomatis infections of humans. The chlamydial diseases for which a vaccine is needed are blinding trachoma and salpingitis. We have previously shown that the immune response to chlamydial infection stimulates both protective and immunopathogenic responses. The antigens that elicit these responses are distinct. These immunologically relevant antigens have been identified and characterized. The protective immune response is mediated by local secretory antibody and is directed against serotype-specific antigenic epitopes located on the major outer membrane protein (MOMP). The deleterious immune response is cell mediated and is directed towards a conformational genus-specific determinant located on a 57K outer membrane protein. The current strategy of this laboratory is to generate recombinant or synthetic immunogens composed of the protective serotype-specific MOMP determinants and test these as vaccine candidates. To this end, the MOMP genes from four C. trachomatis serovars have been cloned, sequenced, and epitope mapped using lambda gt11 subclones expressing antigenic determinants reactive with protective MOMP monoclonal antibodies. These mapping studies have identified both the immunodominant B cell MOMP epitope and a closely associated highly conserved α-helical amphipathic sequence that may be the immunoregulatory T-helper cell determinant. Those regions that encode for the T-helper cell and B cell MOMP determinants will be subcloned into expression vectors, and the recombinant antigens isolated and used as vaccine candidates in chlamydial animal models. Two approaches will be followed for the development of a chlamydial vaccine using recombinant antigen: (i) to prime the hosts immune response with protective recombinant antigens with experimental or natural exposure providing the antigenic stimulus for induction of a protective secondary immune response, or (ii) to employ recombinant antigen to selectively boost protective immunity in animals previously primed by experimental infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00234-06 LMSF

PERIOD COVERED
October 1, 1986, to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biology of Intracellular Parasitism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. Hackstadt	Senior Staff Fellow	LMSF, NIAID
Others:	A. B. Moos	Staff Fellow	LMSF, NIAID
	S. Vishwanath	Visiting Associate	LMSF, NIAID

COOPERATING UNITS *(if any)*
Jim Newhall, University of Indiana; Louis P. Mallavia, Washington State University; and Hubert Mayer, Max-Planck Institut, Freiberg.

LAB/BRANCH
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SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER.
3.4	2.4	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project examines mechanisms of pathogenesis of obligately intracellular bacteria. Studies on Coxiella burnetii, the etiologic agent of Q fever, have continued to focus on the lipopolysaccharides (LPS). Our demonstration last year of structural and/or antigenic variation in the LPS of strains of C. burnetii isolated from chronic Q fever endocarditis vs. acute Q fever has led us to analyze the role of LPS in pathogenesis. Studies in animal model systems have demonstrated that the LPS is a major determinant of virulence affecting both the fever response and ability of the microorganism to persist in the host. Comparisons of the virulence of endocarditis vs. acute type strains of C. burnetii have shown that both are extremely infectious, although the acute type strain has over 100,000-fold greater ability to induce a fever response. Structural analyses have revealed that the wild type LPS sterically blocks access of antibodies to the surface of C. burnetii. Surface proteins are accessible on the avirulent phase of C. burnetii. The role of serum complement in killing of avirulent C. burnetii has been demonstrated, thus providing an explanation for the avirulence of the phase II organisms. The two virulent phases 9mi/I and 9mi/Cr avoid complement killing by different mechanisms. Work with Chlamydia trachomatis has continued to focus on the putative adhesins. Affinity chromatography procedures for the purification of these molecules have been developed and purified proteins used to generate monoclonal antibodies. These antibodies will be used in attempts to molecularly clone the adhesins.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AI 00235-05 LMSF

PERIOD COVERED
October 1, 1986, to August 1, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies on Nonprotein Surface Constituents of Neisseria gonorrhoeae

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. J. Hitchcock	Senior Staff Fellow	LMSF, NIAID
Others:	W. B. Baehr	Senior Staff Fellow	LMSF, NIAID
	T. M. Brown	Microbiologist	LMSF, NIAID
	S. F. Hayes	Bio. Lab. Tech.	LPB, NIAID

COOPERATING UNITS (if any)
John Boslego, WRAIR; Emil Gotschlich, Rockefeller; Keith Joiner, LCI, NIAID; Alf Lindberg, Karolinska Institute, Huddinge, Sweden; Mark Peppler, U of Edmonton; E. N. Robinson, U of Louisville

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.05	1.1	0.95

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Gonorrhea was described in ancient Chinese, Japanese, Egyptian, Greek and Roman literature as well as in the scriptures of the Old Testament. The etiologic agent, a gram-negative bacterium--Neisseria gonorrhoeae, is an obligate pathogen of humans. In the process of evolution, the organism has devised a very successful life style with respect to its human host. Infected humans do not develop an effective immune response to this pathogen, consequently silent, serial, and chronic infections are common. Our ultimate aim is to interfere successfully at some early stage of the disease process; in order to approach this problem, we must consider the biology of the host-parasite relationship. The surface of the gram-negative bacterium, the outer membrane, is a mosaic of tightly associated lipopolysaccharide (LPS) and protein molecules. Additionally, in some organisms, the outer membrane itself is covered by loosely bound polymers which comprise the bacterial capsule. It is the surface of the bacterium which interacts with the host in early stages of parasitism, and it is the host defenses against components of the bacterial surface which play a role in the prevention, resolution or, occasionally, exacerbation of bacterial diseases. We are engaged in the study of the structure and function of nonprotein surface constituents of N. gonorrhoeae. Those neisserial components of particular interest are the common proteolipid (the H.8 antigen), LPS, and the capsular polysaccharide. Our objectives are to isolate and characterize these surface molecules as well as the corresponding structural and regulatory genes in order to determine their role in the pathogenesis of disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00413-04 LMSF

PERIOD COVERED

October 1, 1986, to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetics of Chlamydia trachomatis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	F. E. Nano	Staff Fellow	LMSF, NIAID
Others:	H. D. Caldwell	Research Microbiologist	LMSF, NIAID
	W. B. Baehr	Senior Staff Fellow	LMSF, NIAID

COOPERATING UNITS *(if any)*

Helmut Brade, Borstel Institut fur Medizin, Borstel, FRD
Theresa Joseph, Foundation for Advanced Education in Science
Franco Menozzi, Mons State University, Belgium

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During the last year we have characterized a number of surface antigenic determinants on Chlamydia trachomatis using recombinant DNA and chemical techniques. The gene encoding the major outer membrane protein (MOMP) of C. trachomatis was subcloned into lambda gt11 in order to isolate small DNA fragments that expressed epitopes of the MOMP. The subclones were then subjected to DNA sequencing, and the sequences were aligned with the MOMP gene sequence. In this manner we have been able to identify the regions of MOMP that contain type-, subspecies-, and species-specific epitopes. The genus-specific epitope of Chlamydia--which is located on the lipopolysaccharides--was characterized using a combination of recombinant techniques and chemical analysis. Recombinants that expressed the genus-specific epitope were analyzed to determine the structure of their LPS, and this knowledge aided in the determination of the structure of the chlamydial LPS structure which contains the genus-specific epitope. The nature of the biosynthetic enzyme involved in the expression of the chlamydial genus-specific epitope is currently being analyzed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00441-03 LMSF

PERIOD COVERED
October 1, 1986, to September 30, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Rickettsia rickettsii Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G. A. McDonald	Staff Fellow	LMSF, NIAID
Others:	S. Vishwanath	Visiting Associate	LMSF, NIAID
	R. E. Mann	Bio. Lab. Tech.	LMSF, NIAID

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are interested in the characterization of two proteins of Rickettsia rickettsii, the causative agent of Rocky Mountain spotted fever (RMSF). These proteins with apparent molecular weights of 120 and 155 kilodaltons (Kd) have been shown to be important immunologically and have been implicated as virulence determinants. A monoclonal antibody (Mab) reactive with the 120-Kd antigen was reactive by ELISA with a virulent strain isolated from Montana (Sheila Smith strain), but not with a strain of lesser virulence isolated from North Carolina (Morgan strain). This Mab was capable of passively protecting mice from a potentially lethal challenge of the Sheila Smith strain, but not from challenge with the Morgan strain; a Mab reactive to the 120-Kd protein of both strains did passively protect against both. SDS-PAGE analysis revealed that the 120-Kd protein of the Morgan strain migrated faster than did that of the Sheila Smith strain, while the relative migration of the 155-Kd protein was slower. An N-octyl-β-D-glucopyranoside (OGP) extract of rickettsiae, enriched for the 120- and 155-Kd proteins, was used to immunize guinea pigs. After challenge, guinea pigs which had been vaccinated experienced decreased height and longevity of fever and less severe scrotal reactions as compared to guinea pigs inoculated with buffer alone. A Mab reactive to the 155-Kd protein was administered along with oxytetracycline to guinea pigs infected with R. rickettsii. These guinea pigs had fewer symptoms of RMSF as compared to groups of guinea pigs treated with either water, oxytetracycline, or Mab alone. We recently reported the cloning in Escherichia coli of the gene encoding the 155-Kd protein. A lysate of this recombinant (JM107[pGAM22]) was used to vaccinate guinea pigs; another group of guinea pigs was vaccinated with a lysate of E. coli harboring the vector alone (JM107[pUC8]). The guinea pigs were challenged with viable R. rickettsii. As compared to the guinea pigs which received the JM107(pUC8) lysate, guinea pigs vaccinated with the recombinant lysate (JM107[pGAM22]) had fewer symptoms of RMSF.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00442-03 LMSF

PERIOD COVERED
October 1, 1986, to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunobiology of Guinea Pig Inclusion Conjunctivitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	N. G. Watkins	Staff Fellow	LMSF, NIAID
Others:	H. D. Caldwell	Research Microbiologist	LMSF, NIAID
	A. B. Moos	Staff Fellow	LMSF, NIAID

COOPERATING UNITS *(if any)*
Richard Morrison, Foundation for Advanced Education in Science

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The pathogenesis of recurrent ocular chlamydial infections is thought to be immunologically mediated (i.e., hypersensitivity). We have developed an animal model to study the immunological response to chlamydial antigens during infection and challenge. We have chosen to study the immunopathology of guinea pig inclusion conjunctivitis (GPIC), a Chlamydia psittaci strain which produces both ocular and genital infections in guinea pigs. We have clinically, microbiologically, and histologically characterized primary GPIC and a delayed hypersensitivity response to a chlamydial antigen. The delayed hypersensitivity response occurs only in animals previously infected at a mucosal surface by GPIC agent. The chlamydial hypersensitivity antigen has been identified as a polypeptide with an M_r of 57 kD. We have developed, using the guinea pig animal model, a model for corneal opacity and vascularization. Penicillin treatment during primary GPIC arrest the chlamydial developmental cycle at the reticulate body stage and thus prevents development of infectious particles. These reticulate bodies are maintained within inclusions in the host cell and secrete chlamydial antigens which may lead to more severe conjunctivitis and corneal involvement through host immune mechanisms. This may prove a useful model to study complications leading to blindness in trachoma. We are investigating the role of delayed hypersensitivity in salpingitis and tubal infertility using the guinea pig model. Preliminary results indicate following a primary vaginal or oviductal infection, edema and cellular infiltration similar to that seen in a delayed hypersensitivity response occurs in the oviduct upon challenge with the Triton X-100 extract of GPIC. This animal model of chlamydial caused infertility is being further developed.

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LABORATORY OF PATHOBIOLOGY
Rocky Mountain Laboratories
Hamilton, Montana
1987 Annual Report
Table of Contents

Z01 AI Project Number		Page
Summary		17-1
00071-16	Studies on Pertussigen -- Munoz	17-7
00082-26	Relation of Viruses to the Genesis of Chronic Disorders -- Hadlow (Terminated)	17-8
00201-08	Structural Characterization of Integrated Viral Genomes -- Garon	17-9
00231-05	Biology of Pathogenic Borrelia and Borrelial Infections -- Barbour (Terminated)	17-10
00402-04	Molecular Cloning and Expression of *Bordetella pertussis* Toxins -- Keith	17-11
00480-02	Pathogen-arthropod Interactions of Vector-borne Diseases Affecting Public Health -- Schwan	17-12
00488-01	Ultrastructural Analysis of Antigenic Determinants in Pathogens -- Garon	17-13
00489-01	Purification and Biochemistry of Molecularly Cloned *Bordetella* Proteins -- Keith	17-14
00490-01	Animal Protection Studies Using Synthetic and Recombinant Pertussis Antigens -- Keith	17-15
00491-01	Role of Antibodies of IgE Isotype in Immunologically Mediated Diseases -- Munoz	17-16
00492-01	Molecular Basis for Infection by *Borrelia burgdorferi* -- Schwan	17-17

ANNUAL REPORT
LABORATORY OF PATHOBIOLOGY
ROCKY MOUNTAIN LABORATORIES
HAMILTON, MONTANA
NATIONAL INSTITUTES OF ALLERGY AND INFECTIOUS DISEASES
OCTOBER 1, 1986, to SEPTEMBER 30, 1987

Members of the Laboratory of Pathobiology have attempted during this past year to provide a more detailed molecular characterization of important aspects of host-pathogen interaction. Several medically important systems were carefully dissected using many modern techniques of molecular biology. Significant progress has been made along several fronts to open the possibility of first defining and then exploiting important bio-products in the future production of safe and effective vaccines. New basic information was obtained in several biological systems as well. The broad interaction among members of the laboratory coupled with a strong guest researcher/collaborator program serves to insure that researchers respond effectively to incorporate newly developed experimental approaches and ideas into their own programs. The Laboratory of Pathobiology functions in four major experimental groups. Dr. Claude Garon serves as Acting Chief.

The Molecular Pathobiology Section has continued to focus on the molecular cloning and expression of genes involved in the production of the toxic components of *Bordetella pertussis*. Animal protection studies using both synthetic and recombinant versions of these medically important antigens have begun as well. The S1 subunit of pertussis toxin is an enzymatically active protein that catalyzes the transfer of ADP-ribose from NAD to guanine nucleotide regulatory proteins in eukaryotic cell membranes. Expression of the gene encoding the S1 subunit has been achieved in *Escherichia coli* using vectors containing the transcriptional and translational control elements of the *lac* operon. By employing these recombinant S1 subunit constructs the structure of the S1 subunit as it relates to its physico-chemical and enzymatic properties can be studied. S1 molecules were produced which lacked the C-terminal 48 amino acids including one of the two cysteine residues and a predicted Rossman-type structure. This molecule exhibited increased solubility upon initial isolation when compared to its full-length counterpart, and retained enzymatic activity. The enzymatic activity of this truncated version of S1, like that of the full-length S1 and intact toxin, appears to be dependent upon the presence of a reducing agent. This requirement suggests that the remaining cysteine in the truncated S1 plays a role in the catalytic mechanism. In addition, the S1 subunit in intact pertussis toxin and the recombinant, truncated S1 can be specifically labeled with radioactive NAD in the presence of ultraviolet irradiation. Photo-affinity labeling of the truncated S1 and analysis of the chemical cleavage products have allowed the tentative delineation of a region of the S1 molecule that contains the NAD-binding site. The genes encoding each of the pertussis toxin subunits have been directly expressed in *E. coli* and the recombinant subunits recovered as inclusion bodies from lysed cells. The subunits react in Western blots with monoclonal and polyclonal antitoxin sera. The recombinant S1 subunit has the same enzymatic activities as native toxin. S1 subunits from both sources modify a 41 Kd membrane-associated protein, believed to be the Ni regulatory protein of the adenylate cyclase complex. Serial amino-terminal truncations of the recombinant S1 protein was accomplished by exonucleolytic digestion of the S1 gene. Analyses of these molecules allowed the identification of a specific region of the S1 subunit possessing a major epitope associated with immunoprotection and a site necessary for its enzymatic activities. In addition, a silent copy of the pertussis toxin operon has been identified in *B. parapertussis* and *B. bronchiseptica*. The elucidation of the structural components involved in the biological activities of the S1 subunit will facilitate the development of specifically altered recombinant pertussis toxin molecules that lack enzymatic activity but retain immunogenic activity. These results will permit the design of specifically-mutagenized subunit genes for production of analog toxin proteins that may prove useful as recombinant vaccines against pertussis. Dr. Jerry Keith serves as Acting Head of the Section.

Lyme disease is now the most common arthropod-borne disease in the United States. Members of the Arthropod-borne Diseases Section, under the direction of Dr. Tom Schwan have concentrated their efforts on this and other vector-borne pathogens. Cultivation of *B. burgdorferi*, the Lyme disease spirochete, in the broth medium BSK-II allows for the isolation and growth of this organism from infected animal tissues. However, studies carried out in this laboratory have indicated that changes in surface proteins, infectiousness, and plasmid profile occur as a result of *in vitro* cultivation. While many plasmids (11 in P-0), ranging in size from 4.9 to 49 kb were detectable in early passages, four of them were "lost" by the time cultures were no longer infectious for mice and ultimately only three plasmids remained in the longtime cultured B31 strain. The loss of two plasmids with estimated sizes of 4.9 and 24 kb coincided with the loss of infectivity for white-footed mice. It is possible for these sequences to have been integrated into chromosomal DNA, and thus, still be present in the spirochete. Spirochetes were no longer infectious after 11-15 passages (5.5-7.5 weeks) in BSK-II medium. The reduction in the number of detectable plasmids with the loss of infectivity raises the possibility that gene(s), encoding for components related to infectivity, may be present on one or more of these extrachromosomal elements. The lost plasmids isolated from early passage cultures are being used as DNA hybridization probes to determine more precisely their fate in cultured spirochetes, and as potential markers for infectious strains.

The Immunopathology Section, with Dr. John Munoz as Acting Head, has continued to characterize the immunopotentiating action of small quantities of crystalline pertussis toxin (Ptx) in a mouse model system using the fluorescence activated cell analyzer. BALB/c mice treated with 400 ng of pertussis toxin showed a marked increase in both B- and T-lymphocytes in the blood. This increase was detected as early as 24 hours after injection of the toxin and was detectable for at least nine days. The greatest change was in the percentage of B-lymphocytes in the blood which increased about 200% of normal value one day after injection. This shift in the percentage of B-cells decreased gradually to normal by day 21. This change was not detected in the spleen. In mice immunized with egg albumin (Ea) and Ptx, about 40% of spleen lymphocytes contained IgE on their surface. Ptx alone also increased the percentage of cells containing IgE on their surface, but to a lesser degree. A booster dose of egg albumin, given 21 days after the first infection, induced only a moderate increase in the percentage of IgE containing cells. As little as 1.5 ng of Ptx was necessary to demonstrate an effect. BALB/c and DBA/2J mice showed an increase in IgE containing cells after immunization with Ea and Ptx, but the response was more pronounced in C57Bl/10 SCN mice. The SJL mouse showed only a slight increase in IgE containing cells. The IgE antibody detected in these mice was directed to egg albumin. No antibodies directed against Ptx could be detected. Mutant strains of *B. pertussis* with altered toxin genes were also tested for activity in these assays. Any of the strains in which the gene for the S1 subunit of Ptx was modified did not induce histamine sensitization, leukocytosis, lymphocytosis, or stimulation of the IgE class of antibodies. Strains in which the S1 gene of Ptx was modified failed to induce sensitivity to anaphylaxis to Ea (antigen used to sensitize the mice), while vaccines made with wild strains were highly effective. In the mouse protection test, strains with intact Ptx gene were fully protective. The protection with strains in which the gene for the S1 subunit was modified were not as pronounced.

The Pathobiology Section, headed by Dr. Claude Garon, continues to provide research and research collaboration utilizing modern methods of transmission and scanning electron microscopy to define those structural alterations that are important in the pathological condition. While the techniques of ultramicrotomy, nucleic acid microscopy, molecular cloning and other methods allowing a full range of morphological evaluation continues to be used, special emphasis has been placed on the use of heteroduplex analysis and electron immunomicroscopy. Double-stranded linear plasmids have recently been demonstrated in *Borrelia burgdorferi*, the spirochetal agent of Lyme disease. A 49 kilobase (kb) linear plasmid contained the *ospA* and *ospB* genes, which encode the major outer membrane proteins of strain B31. Molecules of the 49 kb plasmid rapidly reannealed after alkaline denaturation; rapid renaturation was prevented if the 49 kb plasmids were first treated with S1 nuclease. When denatured plasmid molecules were examined directly in the electron microscope, single-stranded circles of approximately a 100 kb were seen. These studies provide direct visual evidence that the linear plasmids have cross-linked ends. This form of DNA occurs in some animal viruses, but it has not heretofore been described in any prokaryotic organisms. Colloidal gold has been used extensively for the precise ultrastructural localization of specific antigens. However, the making of gold colloids, composed of particles of less than 60 Å usually requires the use of white phosphorus and dimethyl ether, a somewhat hazardous process. Through the use of a procedure developed in this laboratory uniform populations of gold particles as small as 20 Å in diameter have been produced and used successfully in electron immunomicroscopy. The technique which makes use of rapid injection and reduced concentrations of $HAuCl_4.3H_2O$ serves to maximize the number of nucleation centers while at the same time reducing the amount of gold available for deposition at each center. The technique is rapid, effective and safe from explosion hazards. It has been used in a broad range of biological systems to demonstrate surface antigen. Preliminary experiments would suggest a utility when coupled with cryoultramicrotomy of localizing internal antigens as well.

ADMINISTRATIVE

Dr. W. J. Hadlow, Research Veterinarian (Pathology) retired in May 1987 after 35 years at the Rocky Mountain Laboratories. Dr. Willy Burgdorfer, Scientist Emeritus of the National Institutes of Health, has remained active and a valuable resource for the Arthropod-borne Diseases Section of the Laboratory.

Guest Researchers in the Laboratory of Pathobiology this past year have included: Dr. Stanley Falkow (Stanford University School of Medicine); Dr. Joseph Guttenplan (New York University); and Kirsten Vadheim (Montana State University). Dr. Witold Cieplak and Dr. Kaarel Kaljot joined the laboratory as NRC and NRSA Researchers respectively. Dr. Yves Lobet joined the laboratory as a Visiting Fellow, Dr. Rex Thomas under the IRTA program and Dr. Doug Brown as a Senior Staff Fellow. Drs. Camille Locht and Kevin Marchitto completed their fellowships during the past year.

Visitors/Collaborators who spent varying amounts of time in the laboratory during the past year included: Dr. Jacques Armand (Institute Merrieux, Lyon, France); Dr. Reno Rappouli (Sclavo Research Center, Siena, Italy); Dr. Neil Burnette (AMGEN); Dr. Luc d'Auriol (Hospital St. Lavis, Paris, France): Dr. James Louie (UCLA Medical Center); Dr. Hardy Chan (Syntex); Dr. Pierre Lallouette (Paris, France); Dr. Joap Nagel (Bit Coeur, The Netherlands); Dr. Pamela Small (Middlebury College): Dr. A. Barnes (CDC); Dr. Jones Clover (Vector Disease Unit, California Department of Health): Dr. Clive Davies (Institute of Virology, Oxford, UK), Dr. Thomas Scott (University of Maryland); Dr. Leonard Mayer (CDC); Dr. Lucy Tompkins (Stanford University School of Medicine); Dr. Kurt Schwinghammer (USDA, Kerrville, TX); Dr. H. Fahrer (Inselspital, Bern, Switzerland); Dr. Paul Lavoie (San Francisco, CA); and Dr. Claus Kindmark, (Kalmar, Sweden).

HONORS AND AWARDS

Dr. Tom Schwan

Invited to organize and chair a symposium on vector competency, 18th Annual Conference of the Society of Vector Ecologists, November 20, 1986, Riverside, CA.

Invited speaker, symposium on spirochetoses, 42nd International Northwestern Conference on Diseases Communicable to Man, August 17, 1987, Ellensburg, WA.

Participant, Third International Conference on Lyme Disease and Related Disorders, September 14, 1987, New York, NY.

Invited to participate in external review of the arborvirus and plague reference and research programs of the Division of Vector-borne Viral Diseases, CDC, Fort Collins, CO, September 29-30, 1987.

Invited to review manuscripts for the Journal of Medical Entomology.

Dr. Jerry Keith

Invited Speaker

Lederle, Pearl River, NY

ASM Meeting, Atlanta, GA

Northwest Dental Association, Missoula, MT

Institute Merrieux, France

Cold Spring Harbor Laboratory

Dr. John Munoz

Reappointed as a staff affiliate of the University of Montana

Trustee of the Stella Duncan Memorial Research Fund, University of Montana.

Invited to review manuscripts for Infection and Immunity

Attended Workshop on Pertussis Vaccine, Washington, D.C. September 1986.

Dr. William Hadlow

Member of Advisory Board, Charles Louis Davis DVM Foundation

Adjunct Professor of Veterinary Pathology, Washington State University

Member of Faculty of Discussants, Davis Foundation

Advisor of Fur Farm Animal Welfare Coalition Ltd.

Dr. Claude Garon

Invited Speaker

Montana Rotary Club Conference

Hamilton Lions Club

Participant in European Congress on Biotechnology 1987 - Amsterdam, The Netherlands

Dr. Willy Burgdorfer

Invited to present lecture at the Annual Meeting of the Swiss Society of Dermatology and Venerology, Basel, Switzerland, October 24-25, 1986.

Invited to speak at the 18th Annual Conference of the Society of Vector Ecologists, Riverside, CA, November 19-21, 1986.

Invited lecturer at Dermatology Clinic, University of Bern, Switzerland, December 4, 1986.

Awarded honorary M.D. degree (Doctor honoris causa medicinae) by Medical Faculty of the University of Bern, Switzerland, December 6, 1986.

By invitation, presented lecture at the Annual Meeting of the Entomological Society of America, Reno, NV, December 7-11, 1986.

Invited to present lecture on Lyme disease at "Allergy and Asthma Symposium," Oshkosh, WI, May 9, 1987.

Invited to present lecture at Marshfield Clinic, Marshfield, WI, May 11, 1987.

Invited to participate and present a lecture at the European Lyme Borrelosis Workshop, Baden, Austria, June 2-4, 1987.

Invited to present lecture at the Dermatology Department, University Hospital, Bern, Switzerland, June 11, 1987.

Invited to present lecture at the Dermatology Department, University Hospital, Zurich, Switzerland, June 22, 1987.

Participated as instructor at the 37th Acarology Summer Program, Ohio State University, Columbus, OH, June 12-July 10, 1987.

Invited to present lecture at the Ohio State Department of Health, Columbus, OH, July 1, 1987.

President of the 42nd International Conference on Diseases in Nature Communicable to Man (INDCNCM), Ellensburg, WA, August 16-19, 1987.

Invited speaker at the Third International Symposium on Lyme Disease, New York, NY, September 14-16, 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 A1 00071-16 LPB

PERIOD COVERED

October 1, 1986, to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on Pertussigen

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John J. Munoz	Acting Head	LPB, NIAID
Others:	M. G. Peacock	Microbiologist	LPB, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Immunopathology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.50	.75	.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Pertussigen (pertussis toxin) [Ptx] is an extremely active toxin affecting various immunological related phenomena. Thus it increases the number of leukocytes, the susceptibility of mice to anaphylaxis and histamine shock; it enhances the induction of experimentally induced autoimmune diseases, has a mitogenic action on T-lymphocytes and enhances markedly the production of IgE and, to a lesser extent, other immunoglobulins as well. In this project, we studied the effect that Ptx has on different lymphocyte populations including the effect on lymphocytes containing IgE on their surface. We found that in the blood, Ptx increased markedly the number of both B- and T-lymphocytes which included the Lyt1+ (helper) and the Lyt2+ (suppressor) lymphocytes. However the most striking effect was the increase in the percentage of B-lymphocytes starting one day after Ptx injection. In the spleen lymphocytes, only a small decrease in the number of B-lymphocytes was observed, although the percentage of all T-cells decreased slightly from the second to the ninth day after Ptx. Ptx induced a marked increase in the IgE containing cells both in the blood and the spleen. This increase was as high as ten-fold that of normal values. With whole cell vaccines made from mutants of *B. pertussis* lacking all or part of the Ptx gene it was found that any modifications of the portion of gene responsible for the production of the S-1 subunit of Ptx made the vaccines inferior in producing lymphocytosis, histamine sensitivity, stimulating IgE or enhancing anaphylaxis. Furthermore, the ability of these vaccines to induce protection against intracerebral challenge was markedly decreased. These studies will be continued during the following year.

LPVD

RMOB

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00082-26 LPB

PERIOD COVERED

October 1, 1986, to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Relation of Viruses to the Genesis of Chronic Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: W. J. Hadlow Research Veterinarian (Pathology) LPB, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Pathobiology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 0.7	PROFESSIONAL: 0.7	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During the past 20 or so years, this project has been concerned largely with two naturally occurring slow viral diseases of animals, namely, scrapie of sheep and goats and Aleutian disease of mink. Emphasis has been on the host-virus interactions that result in the slow evolution of these diseases--polioencephalopathy in scrapie and progressive glomerulonephritis in Aleutian disease. Quantitative virologic information, obtained by endpoint titration in animal assay systems, and morphologic findings have been used to gain an insight into the pathogenesis of each disease. In scrapie, virus replicates for many months mainly in lymphoreticular tissues before reaching the central nervous system where secondary replication gives rise to fatal degenerative neurologic disease. In Aleutian disease, virus replicates early in lymphoreticular tissues, liver, kidney, and intestine. Depending on the strain of virus and genotype of mink, this soon leads to viremia. When it is sustained, virus in the blood forms soluble complexes with specific antibody that become deposited in glomeruli, giving rise to slowly progressive renal disease. Such information on both diseases has implications for understanding broadly comparable human diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00201-08 LPB

PERIOD COVERED

October 1, 1986, to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Characterization of Integrated Viral Genomes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Claude F. Garon	Acting Chief	LPB, NIAID
OTHERS:	Lori L. Petersen	Biologist	LPB, NIAID

COOPERATING UNITS (if any) LMM (Martin, Rutledge) LMO/NCI (T. S. Papas)
CDC (Mayer), Department of Medicine and Microbiology
University of Texas Health Science Center, San Antonio, TX

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Pathobiology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	0.8	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Retroviruses provide a unique and important system for the study of integrative recombination. Retroviral genomes are integrated with high efficiency through a double stranded, circular intermediate at specific sites within the viral genome, but at a large number of sites in the host chromosome. Often a consequence of this integration event is in addition to viral replication, a change in cell growth characteristics. Modern methods of molecular cloning and analysis allow for the detection and amplification of rare DNA sequences such as a single integrated viral segment. Molecular clones of several newly integrated retroviral genomes have been produced in either plasmid or bacteriophage cloning vehicles using recombinant DNA techniques and have been characterized using electron microscope heteroduplex and R-loop methods. Detection of sequence homology even when rearranged or interrupted by intervening cellular DNA is often accurately mappable in the electron microscope using these methods. These studies have not only shown the arrangement of integrated viral sequences within infected host cell DNA, but have also demonstrated the presence and sequence arrangement of certain viral transforming sequences within normal, uninfected host cells as well. These studies have recently been expanded to include a structural determination of the genetic relatedness of AIDS virus isolates from around the world. Unique inverted repeat sequences structurally resembling transposable elements have been identified in the area of retroviral integrations and molecularly cloned for further analysis. The major objective of these studies has been the application of physical and biochemical techniques to assess the influence of integrative position or flanking cellular sequences on subsequent viral function and to define in molecular terms those events which take place during integrative recombination in eukaryotic cell systems.

LPVD

RMOB

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AI 00231-05 LPB

PERIOD COVERED
October 1, 1986, to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biology of Pathogenic Borreliae and Borrelial Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Alan Barbour	Head, Section	LPB, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Pathobiology

SECTION
Arthropod-borne Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00402-04 LPB

PERIOD COVERED

September 30, 1986, to October 1, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Cloning and Expression of Bordetella Pertussis Toxins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Jerry M. Keith, Acting Head, LPB, NIAID

Others: C. Locht	Visiting Fellow	S. Smith	Microbiologist
K. Marchitto	Sr. Staff Fellow	Y. Lobet	Visiting Fellow
K. Vadheim	Guest Worker	D. Brown	Sr. Staff Fellow
M. Szulczynski	Visiting Fellow	L. Mutch	Biologist
K. Kaljot	NRSA Researcher	W. Cieplak	NRC Researcher
All LPB, NIAID			

COOPERATING UNITS (if any)

AMGEN (W. N. Burnette, V. Mar), Thousand Oaks, CA; National Institute of Health (H. Sato), Toyko, Japan

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Molecular Pathobiology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.38	2.1	1.28

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Functional objectives are to investigate genetic molecular structure of pathogens, to define the role of gene products in pathogenic mechanisms and to perform studies directed toward development of vaccines using molecular or synthetic production of immunogenic peptides from microbial agents. Our major emphasis is focused on cloning and expression of genes relevant to the toxic components of B. pertussis. Pertussis toxin is the protective component of the whooping cough vaccine; however its toxin activity may cause the harmful side effects associated with current vaccines. The toxin is composed of five dissimilar subunits; subunit S1 contains an ADP-ribosyltransferase activity responsible for most if not all of the toxin's biological activities. We recently cloned the complete pertussis toxin operon and determined the nucleotide sequence as well as the deduced amino acid sequences of the individual subunits. A patent application for this cloned gene has been filed and several corporations have applied to license the patent for vaccine development. We are using the cloned gene to study the structure/function relationship between pertussis toxin, pathogeneses, and immunoprotection. The genes encoding each of the B. pertussis toxin subunits were directly expressed in Escherichia coli and the recombinant subunits recovered as inclusion bodies from lysed cells. The subunits react in Western blots with monoclonal and polyclonal antitoxin sera. The recombinant S1 subunit has the same enzymatic activities as native toxin; S1 subunits from both sources modify a 41 Kd membrane-associated protein, believed to be the Ni regulatory protein of the adenylate cyclase complex. Serial amino-terminal truncations of the recombinant S1 protein was accomplished by exonucleolytic digestion of the S1 gene. Analyses of these molecules allowed us to identify a specific region of the S1 subunit possessing a major epitope associated with immunoprotection and a site necessary for its enzymatic activities. These results will permit us to design specifically-mutagenized subunit genes for production of analog toxin proteins that may prove useful as recombinant vaccines against pertussis.

LPVD

RMOB

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00480-02 LPB

PERIOD COVERED

October 1, 1986, to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogen-arthropod Interactions of Vector-borne Diseases Affecting Public Health

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Tom G. Schwan	Staff Fellow	LPB, NIAID
OTHERS:	Rex E. Thomas	IRTA Fellow	LPB, NIAID
	Merry E. Schrumpf	Bio. Lab. Tech. (Micro)	LPB, NIAID
	Robert H. Karstens	Bio. Lab. Tech. (Micro)	LPB, NIAID

COOPERATING UNITS *(if any)*

Center for Disease Control, Fort Collins, CO (A. M. Barnes); Stanford University, CA (K. McDonough and S. Falkow); Yale Arbovirus Research Unit, New Haven, CT (D. L. Knudson)

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Arthropod-borne Diseases

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are to develop molecular approaches to investigate pathogen-arthropod interactions of vector-borne diseases of human importance in the United States. The first objective has been to develop a sensitive DNA hybridization probe to specifically identify Yersinia pestis, the causative agent of bubonic plague, in naturally infected fleas. Such a probe will also allow us to examine infection rates in numerous species of fleas held under various ambient conditions and test their competence to transmit this disease. DNA hybridization probes have also been developed for the detection and identification of Borrelia burgdorferi and Borrelia hermsii, the causative agents of Lyme disease and relapsing fever, respectively.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00488-01 LPB

PERIOD COVERED

June 1, 1987, to September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

Ultrastructural Analysis of Antigenic Determinants in Pathogens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Claude F. Garon	Acting Chief	LPB, NIAID
OTHERS:	Stanley F. Hayes	Bio. Lab. Tech. (Micro)	LPB, NIAID
	Dan Corwin	Bio. Lab. Tech. (Micro)	LPB, NIAID
	Willy Burgdorfer	Scientist Emeritus	LPB, NIAID

COOPERATING UNITS *(if any)*

LPVD, RML, NIAID
LMSF, RML, NIAID
Department of Medical Microbiology - Stanford University School of Medicine

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Pathobiology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	0.2	1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project will serve to consolidate and to expand our efforts at identifying, localizing, and characterizing antigens and gene products which are important in host pathogen relationships. While we have used colloidal gold and ferritin labeling to identify important cellular components which are located on the surface of various pathogens, new techniques of cryofixation, cryoultramicrotomy and immuno-labeling will allow precise localization of important antigens which may be located inside the cell. These techniques will allow tracking of antigens through synthesis, processing and genetic control modulation. We have recently developed procedures for laboratory synthesis of uniform, precisely-sized colloidal gold markers which, when coupled with functional and specific antibody molecules, will allow for multiple antigen location and subsequent ultrastructural analysis.

LPVD

RMOB

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00489-01 LPB

PERIOD COVERED

January 1, 1987 to October 1, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Purification and Biochemistry of Molecularly Cloned Bordetella Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Jerry M. Keith, Acting Head, LPB, NIAID

Others:	W. Cieplak	NRC Researcher	C. Locht	Visiting Fellow
	Y. Lobet	Visiting Fellow	L. Mutch	Biologist
	M. Szulczynski	Visiting Fellow	S. Smith	Microbiologist
	D. Brown	Sr. Staff Fellow	K. Kaljot	NRSA Researcher
	S. Northcutt	Biologist	All LPB, NIAID	

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Molecular Pathobiology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.06	2.28	1.78

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Development of new and safer whooping cough vaccines is a major priority of health policy organizations and vaccine manufacturers. Evidence accumulating from clinical trials of acellular pertussis products strongly indicates that pertussis toxin will be a necessary, and perhaps sufficient, component of any new vaccine. Anticipating the need for purer and non-reactogenic reagents, we have cloned and recombinantly expressed the genes for each of the toxin component proteins in Escherichia coli. These nonfusion recombinant polypeptides are synthesized to very high levels as cellular inclusion bodies, greatly facilitating their isolation. This project involves purification and characterization of these protein antigens produced by the host system. In many instances, these expressed proteins represent up to 40% of the total cell protein; however, these proteins are often insoluble and form inclusion bodies in the E. coli cell. Various techniques are being developed to solubilize these proteins. Thus far we have succeeded in purifying various versions of subunits S1, S2, and S4. Manipulation of the gene segment encoding the A protomer (S1 subunit) has permitted identification of regions possessing immunodominant and immunoprotective epitopes and sites critical for its enzymatic activities. We expect that our recombinant reagents will allow us to develop a stable, safe, and efficacious subunit vaccine against pertussis.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00490-01 LPB

PERIOD COVERED

January 1, 1987, to October 1, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Animal Protection Studies Using Synthetic and Recombinant Pertussis Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Jerry M. Keith

Others:	K. Kaljot	NRSA Researcher	LPB, NIAID
	S. Northcutt	Biologist	LPB, NIAID
	W. Cieplak	NRC Researcher	LPB, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Molecular Pathobiology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	0.85	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project involves performing studies directed towards the development of vaccines using recombinant DNA technology to synthesize immunogenic peptides from microbial agents. The objective is to identify specific natural or genetically modified proteins which when used as immunogens will protect against challenge by Bordetella pertussis, the causative agent of whooping cough. These antigens will then be considered for use in a new generation component vaccine. We are presently working on pertussis toxin, a protein consisting of five subunits, which is a major protective immunogen in whooping cough vaccines. Our initial effort involves evaluating individual subunits of pertussis toxin produced synthetically using recombinant DNA technology. Our studies of immunoprotection by recombinant S1, S2, and S4 against lethal intracerebral challenge in mice yielded ambiguous results similar to those found for acellular vaccine materials, raising significant questions about the validity of this potency assay for newer pertussis vaccines. It is clear that new procedures must be developed to evaluate the effectiveness of pertussis antigens to protect against the disease. We are now testing protocols for evaluating potential protective antigens using active and passive immunity. Protection against the following biological effects of the toxin are being evaluated: lymphocytosis, histamine sensitivity, islet activation, CHO cell clumping, hemagglutination, and ADP-ribosytransferase and NAD-glycohydrolase activities.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00491-01 LPB

PERIOD COVERED

October 1, 1986, to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Antibodies of IgE Isotype in Immunologically Mediated Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John J. Munoz	Acting Head	LPB, NIAID
Others:	M. G. Peacock	Microbiologist	LPB, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Immunopathology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.50	0.25	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project will study the role that antibodies of the IgE isotype play in autoimmunity and other immunologically mediated phenomena such as delayed-type hypersensitivity and inflammation caused by antigens in sensitized animals. This important field has not been adequately studied in the past. The project has just been initiated and we are presently developing specific monoclonal antibodies to assay for IgE. Methods for the stimulation of IgE forming cells are being employed to facilitate the fusion of IgE forming cells with SN1 tumor cells to form hybridomas secreting IgE with specificity to selected antigen to be used in our studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00492-01 LPB

PERIOD COVERED

October 1, 1986, to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Basis for Infection by Borrelia burgdorferi

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Tom G. Schwan	Staff Fellow	LPB, NIAID
OTHERS:	Willy Burgdorfer	Scientist Emeritus	LPB, NIAID
	Merry E. Schrumpf	Bio. Lab. Tech. (Micro)	LPB, NIAID
	Robert H. Karstens	Bio. Lab. Tech. (Micro)	LPB, NIAID

COOPERATING UNITS *(if any)*

Southampton Hospital, Southampton, NY (A. MacDonald)

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Arthropod-borne Diseases

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In vitro cultivation of Borrelia burgdorferi, the causative agent of Lyme disease, allows isolation and growth of this bacterium from infected animal tissues. However, continuous cultivation in broth medium (BSK-II) appears to cause a reduction in the number of plasmids and a loss of infectivity for white-footed mice. The reduction in the number of detectable plasmids with the loss of infectivity suggests that gene(s), encoding for components related to infectivity, may be present on one or more of these extrachromosomal elements. The objectives of this project are to (1) determine if genes present on plasmid DNA do control infectivity, (2) characterize these plasmids, (3) clone the infectivity genes and express those components that promote borrelia infection in mammals, and (4) determine immunogenic properties of components responsible for infection.

PHS 6040 (Rev. 1/84) GPO 914-918

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Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
1987 Annual Report
Table of Contents

Z01 AI Project Number		Page
Summary		18-1
00072-16	Role of Host and Viral Factors in Resistance to Rabies Virus Infection in Mice - Lodmell	18-3
00074-15	Genetically Controlled Mechanisms of Recovery from Friend Virus-Induced Leukemia - Chesebro	18-4
00085-10	Pathogenesis of Aleutian Disease Virus Infection - Bloom	18-5
00086-10	Pathogenesis of Diseases Induced by Non-Oncogenic Retroviruses - Portis	18-6
00199-08	Immunobiology of Aleutian Disease - Race	18-7
00260-06	Role of Endogenous and Recombinant Retroviruses in Leukemia and Differentiation - Chesebro	18-8
00262-06	Role of Pentraxins in Acute and Chronic Pathology - Coe	18-9
00263-06	Structure and Function of the ADV Genome - Bloom	18-10
00264-06	Structure and Function of Endogenous Retroviruses Expressed During Differentiation - Portis	18-11
00265-06	Immunobiology of Scrapie Virus Infection - Race	18-12
00266-06	Genetic Structure of Murine Retroviruses - Evans	18-13
00386-03	Transformation of Hematopoietic Cells by Avian Tumor Viruses - Palmieri	18-14
00418-04	Immunobiology of Equine Infectious Anemia Virus, a Retrovirus Model for Aids - Chesebro	18-15
00468-02	Biology of Human AIDS Retrovirus - Chesebro	18-16

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Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1986 to September 30, 1987

RESEARCH HIGHLIGHTS

A cDNA clone of mouse scrapie-associated prion protein gene has been expressed in vitro in eukaryotic cells. No clinical scrapie was induced by inoculation of mice with homogenates of these cells, suggesting that mouse prion protein alone is not the infectious scrapie agent.

Protective immunity to a murine retrovirus was found to be influenced by the K and I-A subregions of the H-2 complex. This effect appeared to act by controlling ability of vaccination to induce virus specific primed T lymphocytes.

Retroviral envelope gene product was shown to have major effect on induction of hemolytic anemia after infection of baby mice with Friend murine leukemia virus.

Monoclonal antibodies specific for the envelope protein of HIV were generated and used to detect infected cells by live cell membrane immunofluorescence.

Retroviral antigenic variants of the lentivirus, equine infectious anemia virus, appeared to be selected in vivo by antibodies reactive with live infected cells.

Neurotropism of murine retrovirus encoded by viral envelope gene. Recombinant retroviruses made inserting the envelope gene of a neurotropic virus into the non-neurotropic Friend virus caused a change in ability to infect CNS cells.

Endogenous mouse gene encoding a unique retroviral envelope gp70 molecule is associated with in vivo resistance to spread of MCF viruses and induction of erythroleukemia.

Complete DNA sequence of Aleutian disease virus of mink reveals open reading frames for non-structural genes.

In vitro cell to cell spread of rabies virus was inhibited by antiviral antibodies. This data eliminated a controversy in the field over whether rabies spread could occur through intracellular contacts similar to Herpes simplex virus.

Polytropic viruses generated after infection with Friend or Moloney viruses are derived from distinct endogenous viral gene sequences.

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Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1986 to September 30, 1987

ADMINISTRATIVE REPORT

The following staff changes occurred at LPVD in the past year: Dr. Byron Caughey from Duke University in Durham, North Carolina, arrived to work as a Staff Fellow. Dr. Masaaki Miyazawa from Sendai, Japan, arrived to work as a Visiting Associate. Dr. Mi Khin Khin Soe arrived from Rangu, Burma to work as guest worker. Dr. Linda Perry from the Department of Microbiology at Emory University Medical School in Atlanta, Georgia, took a position as an Expert. Dr. Marc Sitbon, a Visiting Associate, returned to France to take a position at the Hopital Cochin in Paris, France. Dr. Richard Morrison, a Staff Fellow, took a position in the Laboratory of Microbial Structure and Function at Rocky Mountain Laboratories.

Summer NIH students at LPVD were Joe Ferriter from Amherst College in Amherst, Massachusetts; Jennifer Van Horn from Amherst College in Amherst, Massachusetts; and Nanette Dowling from Montana Tech in Butte, Montana.

Summer student guest worker was Kristi Hurley from Stevenville, Montana.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00072-16 LPVD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Host and Viral Factors in Resistance to Rabies Virus Infection in Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. L. Lodmell	Scientist Director	LPVD, NIAID
Others:	None		

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The principal objective of these studies is to determine host and viral factors that influence genetically-controlled murine resistance to, and recovery from street rabies virus (SRV) infections following viral invasion of the central nervous system. In vitro studies have shown that the cell-to-cell spread of three different rabies viruses in neuronal and non-neuronal cells was inhibited by $\geq$ 75% with less than one international unit/ml of human antirabies immune globulin. In contrast, only small foci of infection developed in monolayers incubated with antibody. Virus persisted in these few cells for > 2 weeks, but spread rapidly to uninfected cells after antibody was removed. Continuing pathogenesis studies have determined that high concentrations of SRV and interferon (INF) were present in the spinal cord (SC) and brains of susceptible A/WySn/J mice, whereas minimal amounts of INF and virus were present in similar tissues of resistant SJL/J mice. Thus, there was a direct correlation between the amount of INF and virus in SC and brains, but no correlation between resistance of SJL/J mice to ascending viral infection in the CNS and the local or systemic expression of INF. Cellular immunity studies have progressed with the establishment of a new and different (SJL/J $H\text{-}2^s$) target cell (SSSV) which is easily infected and then lysed with rabies specific antibody and complement. Target cells for use with highly susceptible A/WySn/J($H\text{-}2^a$) and the moderately susceptible C57Bl/6/J($H\text{-}2^b$) mice have been identified. The importance of cytotoxic T cells in resistance to SRV are in progress. The importance to T-cells and B-cells in resistance to SRV also are being studied through ablation of the immune response with cyclophosphamide and cyclosporin A (CS-A). Preliminary experiments with CS-A have shown that immunosuppressed SJL/J mice die within four days of virus injection. The importance of T-independent and T-dependent immune responses in resistance to SRV, as identified by the use of these drugs, and the passive transfer of immune cells, serum, and/or monoclonal antibodies is under investigation.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00074-15 LPVD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Genetically Controlled Mechanisms of Recovery from Friend Virus-Induced Leukemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	B. Chesebro	Chief	LPVD, NIAID
Others:	M. Miyazawa	Visiting Associate	LPVD, NIAID
	R. Morrison	Staff Fellow	LPVD, NIAID

COOPERATING UNITS (if any)
Drs. B. Moss and P. Earl, LVD, NIAID, Bethesda, MD

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Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.2	0.6

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- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The main aim of this work is study of genetically controlled host defense mechanisms involved in protective immunity and recovery from Friend retrovirus complex (FV)-induced erythroleukemia in mice as a model for human retrovirus diseases such as AIDS and leukemia.

We have continued previous studies of the influences of H-2 genotype on both FV-induced immunosuppression and protective immunity against FV. Initial results suggested that mice with a high susceptibility to retrovirus induced immunosuppression could not be protected by immunization with a vaccinia recombinant expressing the FV envelope gene product. However, use of H-2 recombinant mice indicated that protective immunity was influenced by the H-2K and I-A subregions, whereas immunosuppression was influenced by the H-2D subregion. Thus, protective immunity was induced even in mice with high susceptibility to FV-induced immunosuppression. These results suggest that humans who are genetically more susceptible to the immunosuppressive effects of HIV infection might still be protected from AIDS with appropriate vaccines.

More recent studies on the mechanism of protection in this system indicate that vaccination induces FV-specific T lymphocyte priming. Subsequent exposure by challenge with FV induces cytotoxic T lymphocytes and a secondary humoral antiviral antibody response, both of which may be involved in recovery from disease.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00085-10 LPVD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis of Aleutian Disease Virus Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. E. Bloom	Medical Officer	LPVD, NIAID
Other:	S. Alexandersen	Visiting Fellow	LPVD, NIAID
	R. E. Race	Research Veterinarian	LPVD, NIAID

COOPERATING UNITS *(if any)*

None

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.2	0.5

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The scope of this project is the study of infections of mink with the Aleutian disease of mink parvovirus (ADV). In the past year we have begun the analysis of ADV infections utilizing strand-specific in situ molecular hybridization. The rationale is based on the fact that replicative forms of ADV DNA (RFs) contain "+" sense nucleic acid strands, whereas virion DNA is largely "-" in sense. Thus, probes that are "-" in sense will preferentially define sites of replication, whereas "+" sense probes will in addition delineate sites where virus is being sequestered. We have applied these probes in in situ hybridization to date on ADV infected tissue culture cells in vitro and on sections of newborn mink with ADV interstitial pneumonitis. Throughout permissive in vitro infection, duplex RFs were localized to the cell nuclei. Virion DNA and capsid proteins were found in the nuclei early in infection, but were later translocated to the cytoplasm. The autoradiographic grains were clearly localized over the infected cells, and the number of cells positive for ADV antigen was the same as that positive for viral DNA, suggesting that in this permissive system, all cells containing viral DNA also expressed viral proteins. In ADV infected neonatal mink, cells replicating ADV were found primarily in the lung. RFs and ADV antigens were found predominantly in the surfactant producing alveolar type II cells, and the pattern was similar to that observed for the tissue culture cells. This observation suggested that the neonatal pneumonitis results from a direct cytopathic infection by ADV of the type II cells, with subsequent depletion of pulmonary surfactant. Viral DNA was also found in other organs, but the strand specific probes made it possible to show that most of this DNA represented virus that was being sequestered in phagocytic elements of the lymphoreticular system. Virion DNA, ADV antigen and mink IgG were found in the histologically normal glomeruli of these infected neonates, suggesting an early form of immune complex nephritis in which intact virions participated.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZOI AI 00086-10 LPVD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis of Diseases Induced by Non-Oncogenic Retroviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. L. Portis Medical Officer LPVD, NIAID

Others: None

COOPERATING UNITS *(if any)* Dr. W. G. Stroop and Dr. J. J. Townsend, Neurovirology Research Lab, Univ. Utah Sch Med, Salt Lake City, UT; Dr. Richard Ziegler, Univ. Minn. Sch Med, Dept. Microbiology, Deluth, MN; Dr. Linda Perry, Dept. Micro. & Immunol., Emory Univ., Atlanta, GA.

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TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.7	0.7	1.0

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☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It is now well documented that several human retroviruses including HIV can induce central nervous system diseases with clinical manifestations ranging from subtle personality, perceptual motor and memory disorders to frank dementia. In addition, HTLV I appears to be the causative agent of tropical paraparesis a devastating motor disease encountered in Africa. It is clear that these viruses replicate in the CNS and for HIV at least, the cell types which are infected have been identified. We are studying a neurovirulent retrovirus of mice which, although only distantly related to the human retroviruses, induces a disease with manifestations which in some respects resemble those of the human diseases. Our primary goals have been to identify the important target cells in the CNS the infection of which leads to clinical disease and to determine the nature of the cytopathology that ensues upon such infection. Like HIV infection, clinical signs of neurologic disease were found to be unaccompanied by any significant histopathology. This has suggested to some investigators that infection of endothelial cells in the CNS may itself mediate the CNS dysfunction. However, we have found no correlation between replication of murine retroviruses in CNS endothelial cells and neurologic disease. Disease was clearly associated with the capacity of WM-E to spread to the parenchyma of the CNS, and, using molecular constructs between the neurovirulent and a non-neurovirulent viruses, extra-vascular spread was found to be mediated by the viral envelope gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00199-08 LPVD

PERIOD COVERED

October 1, 1986, to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunobiology of Aleutian Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. E. Race	Research Veterinarian	LPVD, NIAID
Others:	M. E. Bloom	Medical Officer	LPVD, NIAID

COOPERATING UNITS *(if any)*

None

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

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☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Monoclonal antibodies were used to study antigenic differences among strains of ADV and to characterize viral proteins in vitro and in vivo. Highly virulent Utah I ADV was clearly delineated from the tissue culture-adapted avirulent ADV-G strain. This specificity could be demonstrated by indirect immunofluorescence (IFA) against infected cultures of Crandell feline kidney cells or against tissues of Utah I ADV-infected mink. Immunoprecipitation analyses utilizing various mAbs identified specific antigenic determinants. When immunoprecipitation-defined reactivities were correlated with IFA tissue and in vitro patterns of reactivity it was apparent that the virus-associated antigenic determinants recognized in vivo were proteolytic products of viral structural proteins. Thus, intact structural ADV proteins were not identified in vivo. However, structural proteins were detected in vitro when ADV-G or Utah I ADV-infected CRFK cells were analyzed.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00260-06 LPVD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Role of Endogenous and Recombinant Retroviruses in Leukemia and Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Chesebro	Chief	LPVD, NIAID
Others:	M. Sitbon	Visiting Associate	LPVD, NIAID
	L. Evans	Senior Staff Fellow	LPVD, NIAID
	F. Hayes		LPB, NIAID

COOPERATING UNITS *(if any)*

Dr. L. D'Auriol, Hopital St. Louis, Paris, France

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	0.3	0.8

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☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The main objective of this project is study of mechanisms of retrovirus-induced pathogenesis. We have been studying anemia and erythroleukemia induced by neonatal inoculation of mice with Friend murine leukemia helper virus (F-MuLV). This disease has two separate pathogenic phases both resulting in anemia and splenomegaly. In the first phase virus infection of erythroid precursors causes premature destruction of erythrocytes, giving a hemolytic anemia; splenomegaly results from compensatory erythropoiesis. The second phase consists of erythroleukemia with splenomegaly and anemia due to lack of production and differentiation of erythrocytes. Using two virus variants differing in virulence it was shown that the viral envelope gene was primarily responsible for the induction of early severe hemolytic anemia, whereas the viral LTR region had the main influence on latency of infection of erythroleukemia. Current efforts are aimed at determining the precise gene sequences responsible for each of these effects.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00262-06 LPVD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Pentraxins in Acute and Chronic Pathology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. E. Coe	Medical Officer	LPVD, NIAID
Others:	None		

COOPERATING UNITS *(if any)*

See attached page

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have been developing various experimental models in hamsters. Our primary interest is female protein (FP), a serum pentraxin which is under sex steroid control in the Syrian hamster. Although FP is very similar structurally to human homologs [serum amyloid P-(SAP) and C-reactive protein (CRP)], this protein as expressed in Syrian hamsters has a number of unique features. However, even in related hamsters (Turkish, Armenian, Dunzgarian) FP expression is under different control than in Syrian, although the protein appears to be very similar structurally. In fact, so similar that hybrid pentameric FP molecules have been constructed in vitro. Thus, in Armenian hamster, FP is not sex limited as male-female serum levels are similar; in contrast to Syrian hamsters, Armenian FP hepatic synthesis is not suppressed by testosterone, although exogenous estrogens, including DES, will down regulate synthesis. Estrogens have another effect on Armenian hamster liver, and that is an acute toxicity which is clinically manifest as a profound jaundice which is detectable within two days of estrogen administration. Of particular interest is the finding that concomitant administration of tamoxifen, an estrogen receptor blocking agent, will inhibit this hepatotoxic effect of estrogens, indicating an estrogen receptor is mediating this liver injury. The role of FP in this hepatotoxicity is unknown, although, low FP levels are only found in those two species which are susceptible the Armenian and Chinese hamsters.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00263-06 LPVD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Function of the ADV Genome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. E. Bloom	Medical Officer	LPVD, NIAID
Others:	Soren Alexandersen		

COOPERATING UNITS *(if any)*

None

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	0.9	1.0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is the study of genome structure and function of the Aleutian disease of mink parvovirus (ADV). In the past year we have completed sequencing the ADV-G genome and found that its overall structure resembles that of other nondefective parvoviruses. A portion of the "left-hand" open reading frame (ORF) has been expressed in bacteria and shown to code for the 71,000 dalton nonstructural protein of ADV. Sequence heterogeneity for the structural protein region of the ADV genome has been demonstrated and the heterogeneity observed might reflect host range determinants involved with pathogenicity. Finally, study of the ADV transcription program has been initiated. Three major polyadenylated RNAs have been detected; 4.5 kb, 2.6 kb, and 0.9 kb.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00264-06 LPVD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of Endogenous Retroviruses Expressed During Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. L. Portis	Medical Officer	LPVD, NIAID
Others	R. Buller	Staff Fellow	LPVD, NIAID
	B. Chesebro	Chief	LPVD, NIAID

COOPERATING UNITS (if any)

Dr. M. Sitbon, Hopital Cochin, Paris, France; Dr. Gary Van Zant, Dept. Molec. & Cell Biology, Texas Tech Univ., Lubbock, TX

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Murine leukemias are studied both for their use in understanding basic mechanisms of neoplasia and to identify potential host factors which participate in disease resistance. Both of these avenues have been fruitful in understanding the factors which control the expression of human cancers. We are studying a genetic locus in the mouse which restricts, in vitro the replication of a class of recombinant retroviruses implicated in the induction of F-MuLV-induced erythroleukemia. This locus encodes two allelic endogenous retroviral gp70 molecules which are differentially expressed in different mouse strains. Our immediate goal has been to determine whether this gene participates in disease resistance in vivo and to identify the cells which express these env gene products. Backcross analysis between leukemia susceptible (IRW) and resistant (DBA/2) strains revealed that progeny receiving the endogenous gp70 gene from DBA/2 mice exhibited a strong resistance to recombinant MCF virus expression in the spleen after F-MuLV inoculation and a distinct 1 to 2 month delay in the tempo of erythroleukemia development. Previous correlative studies revealed that this gp70 gene is expressed on a population of erythroid precursors present in the early murine fetal liver and are now using cell sorting techniques to characterize these cells functionally. In addition, progress is being made toward the ultimate goal of cloning these genes.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00265-06 LPVD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunobiology of Scrapie Virus Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. E. Race	Research Veterinarian	LPVD, NIAID
Others:	B. Caughey	Staff Fellow	LPVD, NIAID
	B. Chesebro	Chief	LPVD, NIAID
	J. Keith	Senior Staff Fellow	LPB, NIAID

COOPERATING UNITS *(if any)*

Dr. A. Haase, Chief, Dept. Microbiology, University of Minnesota, Minneapolis, MN; Dr. M. Buchmeier, Dept. of Immunology, Scripps Clinic and Research Foundation, La Jolla, CA.

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NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.6	1.9	1.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Scrapie is a naturally occurring spongiform encephalopathy of sheep and goats which causes clinical and pathological changes similar to those of Creutzfeld-Jakob and Kuru diseases of man. A unique protein called prion protein (PrP) has been found to be a major component of purified samples of scrapie infectivity and is believed by some people to be the actual infectious agent. We previously isolated two cDNA clones of the PrP mRNA from scrapie-infected mouse brain. We have ligated the cloned mouse PrP cDNA into an expression plasmid containing bovine papilloma virus (BPV) and have induced PrP synthesis in mouse epithelial cells. To test the possible infectivity of these expressed proteins, sonicated cell suspensions were inoculated intracerebrally into weanling RML mice. By 160 days post-inoculation no mice had developed clinical signs of scrapie suggesting that PrP may not be the infectious scrapie agent, but is more likely to be a normal cellular protein which accumulates in aggregated forms as a result of the disease process. The possibility that post-translational modification of the endogenous gene product results in creation of the infectious scrapie agent has not been completely excluded.

Because animal tissues have not provided satisfactory substrates for the biochemical analysis or isolation of the scrapie agent, we have continued to work on development of scrapie infected tissue culture systems. Toward this goal neuroblastoma cell lines of mouse origin were successfully infected with scrapie agent. Infected cultures were maintained through 21 passages, well beyond the number needed to assure that replication had occurred. Infection appeared to be species-specific in that agent derived from hamster brain did not infect the mouse derived neuroblastoma cell lines. Positive clones have now been derived from these original cultures. They contain approximately 50-100 fold more agent than was found in the original cultures and should provide a new, more homogeneous source of scrapie agent.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00266-06 LPVD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic Structure of Murine Retroviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. H. Evans	Senior Staff Fellow	LPVD, NIAID
Others:	J. D. Morrey	Staff Fellow	LPVD, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Genetic alterations of retroviruses have been documented with lentiviruses such as EIAV and the AIDS virus, as well as with murine leukemia viruses (MuLVs). Ecotropic MuLVs undergo recombination with gene sequences of the mouse to generate recombinant viruses termed polytropic or mink cell focus-forming (MCF) viruses, which have frequently been implicated as the proximal agents involved in transformation to malignancy. A major goal of this project is to characterize the recombinant MuLVs and to define their role in disease induction. Initial studies demonstrated that ecotropic viruses which induce distinct types of leukemias (i.e., erythroleukemia vs. lymphocytic leukemia) recombine with distinct mouse genes to generate MCF viruses. We have extended these studies using a novel retrovirus assay to define new major populations of recombinant viruses which were undetected using conventional assays. The new recombinants isolated during erythroleukemia induction exhibited distinctly different infectious properties than those isolated during lymphocytic leukemia induction and, in both cases, represented the overwhelming majority of recombinant viruses present. Additional studies on tissue-specific replication of ecotropic viruses indicated that the site of replication is controlled by multiple sequences of the viruses, and that leukemia induction requires a high level of replication in the oncogenic target tissue only transiently, early after infection.

AKR mice exhibit a high level of spontaneous leukemia and express oncogenic recombinant viruses immediately preceding the earliest incidence of leukemia. The recombinants generated in AKR mice are derived by a stepwise recombination mechanism which is not fully understood. We have identified and studied the structure and expression of a new virus in AKR mice which appears to serve as a recombination intermediate in this stepwise process. Our findings have enabled us to arrive at a detailed model for the generation of the oncogenic recombinants.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00386-03 LPVD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Transformation of Hematopoietic Cells by Avian Tumor Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S. Palmieri Senior Staff Fellow LPVD, NIAID

Others: None

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00418-04 LPVD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunobiology of Equine Infectious Anemia Virus, a Retrovirus Model for AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Chesebro	Chief	LPVD, NIAID
Others:	S. Carpenter	Staff Fellow	LPVD, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The morphological, genetic, and serological relatedness between equine infectious anemia virus (EIAV) and human immunodeficiency virus (HIV) has increased interest in understanding the pathogenesis of EIA, the mechanisms of viral persistence and the host response to infection. The goal of this project is to elucidate factors important in the generation and selection of genetic and antigenic variants of EIAV with regard to the role of variation in lentiviral persistence and pathogenesis. We previously demonstrated that variant-specific epitopes are expressed on EIAV-infected cells and suggested that specific elimination of certain variant-infected cells might be a critical immune response in the early selection of viral variants. Biochemical identification of viral proteins expressing variant-specific epitopes was assayed by radioimmunoprecipitation using detergent lysates of EIAV-infected cells. However, viral antigens exposed in detergent lysates were highly cross-reactive whereas antigens exposed on the surface of live cells were strain-specific. Moreover, antibody to the broadly reactive epitopes was able to prevent detection by immunoprecipitation of antibody to more biologically interesting, strain-specific epitopes. Thus, immunoassays which rely on detergent treatment of antigen are unlikely to detect strain-specific antibody with potential biological importance in variant selection.

We have succeeded in the molecular cloning of the MA-1 isolate of EIAV from a circular, replicative form of viral DNA. The availability of a molecularly cloned field isolate of EIAV provides a broader base from which to study the extent and location of variation among antigenically distinct isolates of EIAV.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00468-02 LPVD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Biology of Human AIDS Retrovirus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Chesebro	Chief	LPVD, NIAID
Others:	None		

COOPERATING UNITS *(if any)*

Dr. T. Folks, LIG, NIAID, Bethesda, MD; Dr. B. Moss, LVD, NIAID, Bethesda, MD

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.2	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The primary goal of this project has been to develop an enumeration assay for infectious HIV. In an initial phase we generated a series of monoclonal antibodies in mice which recognize the HIV envelope protein. The preliminary results indicate that the antibodies will detect HIV antigens on live, infected cells as well as methanol-fixed, infected cells by indirect immunofluorescence. Current studies are in progress to determine which of the two envelope glycoprotein components are recognized by these monoclonal antibodies. Subsequently the antibodies will be used to examine whether HIV infected cells present in blood of AIDS patients can be detected using FACS analysis.

We are also continuing our efforts to develop a sensitive cell line which can be used in assays for infectious HIV. Approaches include fusion of sensitive suspension cell lines to cell lines capable of attaching to plastic surfaces as well as transfer of CD-4 genes into various attached cell lines. These cell lines are now being screened for their ability to be infected _in vitro_ by HIV.

RMOB

ROCKY MOUNTAIN OPERATIONS BRANCH
Rocky Mountain Laboratories
Hamilton, Montana
1987 Annual Report
Table of Contents

Introduction 19-1

General Overview 19-2

Annual Report
Operations Branch
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1986, to September 30, 1987

Introduction

The branch provides all services necessary to the professional staff in the pursuit of their investigations. Fiscal support includes budget management, procurement and initiating payments and follow up on financial obligations related to purchases, contracts, staff and official guest travel and expenses for conferences held at RML. Other support covers the following areas: personnel, communications, library services, secretary backup service, grounds care, custodial, security, media preparation, waste disposal including hazardous wastes and radioactive wastes, glassware cleaning, photography, animal rearing and care, motor pool, operation of power plant and full maintenance and minor laboratory renovations in every area except electronics.

In late 1985 the Director, IRP, NIAID, asked if it would be possible to rear research animals at the RML for use on the Bethesda campus. It was felt that this would be quite feasible and with the assistance of Dr. Peter Golway, the project was initiated. Forty-two breeding pairs from three strains were received in April and May 1986, and we began to build up the breeding colonies in the rearing unit of the Animal Care Section. The first mice for research use on the Bethesda campus were shipped last December. Currently we are producing three strains of mice (BALB/c ANN, C-57BL/6N, C57B10ASGSN/J) and are shipping about 1,200 - 1,300 mice per month. The mice have been found to be of very acceptable quality for research.

Last October it was decided that the operation of the glassware unit should be contracted out to private enterprise. Work was initiated to acquire a contract, and five proposals have been received. It is anticipated that the contract will be awarded in late September of this fiscal year.

The Maintenance Section has performed several renovation projects for the Laboratory of Persistent Viral Diseases during the past year. To date, this has included two offices and five laboratories. In addition, another autoclave facility is being constructed and work will soon begin to convert two old storerooms into laboratories. Work is also commencing on converting the old incinerator building into a new carpenter shop.

General Overview of the Responsibilities of Operations Branch

The fiscal and procurement department manages a budget of nearly $2,000,000 Payroll is not included in this figure. It covers only the purchase of services, supplies, and equipment used in the operation of the laboratories. Timekeeping and submission of the payroll are also handled in this unit.

Personnel handles all actions and advises on personnel matters. This department is also charged with operation of the Job Training Partnership Act in association with the local Montana State Employment Office. However, because of budget constraints to the state on this program, it was only active for a short time this past year. Also handled by Personnel are persons under the following programs: Stay-in-School, Summer Aides, Work Study, Student Volunteers, Visiting Program, and students studying for advanced degrees.

Most of the biological media used in the research laboratories is prepared in a special laboratory by a technician. Glassware is cleaned and sterilized in the glassware department for reuse in the laboratories.

The Graphic Arts Department provides full professional services necessary in the laboratories with the exception of medical artistry.

The library provides a full range of services for the RML staff, i.e., selections and acquisitions, cataloging, circulation, inter-library loans, reference and bibliographic services, computerized data base searches, and preparing periodicals for binding.

The Animal Unit raises guinea pigs, 17 strains of mice, 5 strains of hamsters, and a colony of microtus. They breed and raise approximately 103,000 animals a year. An additional 2,100 animals are purchased annually from outside sources, including mink, rabbits, mice, chickens and hamsters. After rearing, care is provided for these animals while they are under experiment.

The Chief of the Branch is responsible for labor management work and administering the technical aspects of the A-76 contracts for Security, Custodial and Operation of the Power Plant with the respective private contractors. Security is provided by a guard on duty every night and all day on weekends and holidays. Custodial services are provided in five laboratory buildings daily except weekends and holidays. Power plant operation provides heat, steam, compressed air, vacuum and emergency power to the entire laboratory complex.

The maintenance department provides repair, service and renovation work in plumbing, electrical, sheet metal, carpentry, air conditioning and refrigeration, including ultra low temperature boxes. With the exception of electronic work, all maintenance is done by the staff. Also provided are demineralized and distilled water. A motor pool consisting of 10 vehicles is maintained. Grounds care including snow removal is provided.

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